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## Antiobesity Effect of Peripheral Exendin-4 and/or Exercise in High-Fat Diet-Induced Obese C57bl/6 Mice

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**Abstract:** This study was conducted to examine the antiobesity effect of exendin-4 and/or exercise treatment in high-fat diet-induced obese C57BL/6 female mice and to evaluate whether these interventions could improve glucose and lipid metabolic profiles, and glucagon-like peptide-1 (GLP-1) secretion. Following the 13 weeks fattening period on the high saturated fat diet (HSFD), diet-induced obese mice continued feeding on the same HSFD and were assigned to four groups (n = 6/group); Control-Saline-Sedentary (C-Sal-Sed), Exendin-4-Sedentary (Ex-4-Sed), Saline-Exercise (Sal-Exr) and Exendin-4-Exercise (Ex-4-Exr). Mice in the exercise groups performed 13 weeks of running on a treadmill. Mice in the exendin-4 groups were injected a 100  $\mu$ L exendin-4 (1 nmol/kg body weight) intraperitoneally once a day. Glucose and lipid profiles, and GLP-1 and Exendin-4 levels were determined. It was observed that sustained exposure of mice fed the HSFD for 26 weeks to exendin-4 and/or five days a week to exercise for 13 weeks resulted in a marked ( $p < 0.001$ ) reduction in gained body weight in Ex-4-Sed, Sal-Ex, Ex-4-Exr groups as compared to the C-Sal-Sed group (28.54, 30.0, 29.78 gm, respectively,  $p < 0.001$ ). This improved hypertriglyceridemia ( $p < 0.001$ ), deterioration in glucose tolerance ( $p < 0.001$ ), and feed efficiency ( $p < 0.001$ ). GLP-1 levels were elevated significantly in Ex-4-Sed, Sal-Ex, and Ex-4-Exr groups as compared to the C-Sal-Sed group (12.17, 11.31, 12.57 and 6.39 pmol/l; respectively,  $p < 0.001$ ). We concluded that exendin-4 and/or exercise are effective treatments and can reverse dyslipidemia and hyperglycemia by inducing a sustainable loss in body weight resulting from long-term feeding of HSFD and increasing serum GLP-1 levels. Early interventions of exendin-4 and/or exercise could play a vital role in modifying physiological pathways, both at the metabolic and endocrine levels.

**Key words:** Exendin-4, glucagon-like peptide-1, high fat diet, exercise, obesity

### INTRODUCTION

The marked increase in the prevalence of overweight and obesity in several developed and developing countries has been described as a global pandemic. In 2010, overweight and obesity were estimated to cause more than 3.4 million deaths worldwide. Globally, Ng and colleagues have found that the proportion of adults aged 20 years or above who were overweight or obese (body-mass index (BMI)  $\geq 25$  kg/m<sup>2</sup>) has increased between 1980 and 2013 from 29.8 to 38.0% in women and from 28.8 to 36.9% in men. Furthermore, the prevalence of overweight or obesity in developed countries has increased considerably in children and adolescents aged less than 20 years; 23.8% of boys and 22.6% of girls in 2013. Similarly, the prevalence of overweight and obesity in developing countries has increased substantially in children and adolescents, from 8.1 to 12.9% of boys and from 8.4 to 13.4% of girls (Ng *et al.*, 2014).

Obesity develops from a chronic failure of the body-weight control mechanisms due to a complex interplay of genetic and environmental risk factors, mainly passive food overconsumption of caloric-dense diets and

sedentary state acting through mediators of energy intake and energy expenditure (Al-Domi, 2014). Furthermore, hormones released from the gastrointestinal tract or adipose tissue including glucose-dependent insulinotropic protein (GIP), glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2, ghrelin, peptide YY and leptin hormones are implicated in body-weight control mechanisms due to their effect on either food intake as satiety or hunger signals or insulin secretion to maintain glucose homeostasis (Mahmud and Al-Domi, 2014; Bloom *et al.*, 2008).

Incretins are gut hormones secreted from enteroendocrine cells in response to eating and regulate the amount of insulin secreted. GIP and GLP-1 are two incretins that share many common actions in the pancreas, yet have distinct actions outside of the pancreas. GLP-1 is released from the gut in response to food consumption and functions as a satiety signal; it stimulates insulin secretion in a glucose-dependent manner (Bloom *et al.*, 2008; Baggio and Drucker, 2007). Exendin-4 is an incretin mimetic, dipeptidyl peptidase IV-resistant potent GLP-1 receptor agonist. It exerts

insulinotropic effects and has multiple glucose regulatory functions through GLP-1 activation receptor. Treatment with exendin-4 increases neogenesis, proliferation and beta cells survival through activation of protein kinase A and the Akt serine-threonine kinase with associated gene expression (Baggio and Drucker, 2007). Furthermore, treatment with exendin-4 reduces food intake, slows gastric emptying and increases satiety (Xu *et al.*, 2006). Exendin-4 enhances insulin sensitivity, glucose transport, and glucose and lipid metabolism by increasing the expression of insulin receptor beta, insulin receptor substrate-1 and glucose transporter 4 in adipocytes, independent of insulin clearance (Barrera *et al.*, 2009).

Sedentary lifestyle has become more prevalent over the past few decades due to changing lifestyle. Exercise is one of the most common prescribed measures not only to prevent weight gain and to induce a subsequent weight reduction via creating a negative energy balance, but also to improve metabolic profile through controlling appetite (Martins *et al.*, 2008). Furthermore, exercise induces significant elevation in GLP-1 levels as compared to sedentary lean individuals (Mahmud and Al-Domi, 2014). Acute exercise led to a significant increase in GLP-1 levels in circulation and to a significant decrease in hunger resulting in a significant reduction in relative energy intake (Dardevet *et al.*, 2004). Hence, the objectives of this study were to investigate the potential antiobesity effect of peripheral exendin-4 and/or exercise in high-fat diet-induced obese C57BL/6 female mice, and to evaluate whether these interventions could improve glucose and lipid metabolic profiles as well as GLP-1 levels.

## MATERIALS AND METHODS

**Experimental animals and diets:** All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Pharmaceutical Research Unit-Royal Scientific Society (PRU-RSS), Amman, Jordan and the Deanship of Academic Research, the University of Jordan, Jordan, and were carried out in conformity with the recommendations of the guide for the care and use of laboratory animals (Clark *et al.*, 1996). Twenty-four pathogen-free female C57BL/6 mice were purchased at 6 weeks of age and an average body weight of 14.5 gm from Harlan Laboratories (Montreal, PQ, Canada). On arrival at the facility, mice were housed individually in specific pathogen-free cages in the PRU-RSS and were maintained at 21 to 25°C and 40 to 60% relative humidity under a light-dark (12:12) cycle in polypropylene cages and stainless steel wire lids. Dirty cages and beddings were cleaned weekly. The specific pathogen-free health status of mice in this facility was monitored. Mice were tested routinely and remained negative for common mouse parasites and pathogens. Prior to the initiation of exercise regimen, mice were fed

the high saturated fat diet (HSFD; 45% kcal fat; 35% carbohydrate, 20%; 4.7 Kcal/gm) for 13 weeks (Research Diets, Inc. (Lot. no. 10021107A8, 2010, USA) and water were provided *ad libitum*.

**Experimental protocol:** Following the 13 weeks fattening period on the HSFD, diet-induced obese mice continued feeding on the same HSFD and were assigned to four groups (n = 6/group); group (1) Control-Saline-Sedentary (C-Sal-Sed), group (2) Exendin-4-Sedentary (Ex-4-Sed), group (3) Saline-Exercise (Sal-Exr) and group (4) Exendin-4-Exercise (Ex-4-Exr). Mice in the exercise groups were subjected to a moderate intensity exercise regimen that lasted for 13 weeks at 30 min per day for five days a week on a six-lane motorized treadmill (Columbus Instruments, Columbus, Ohio-U.S.A) at a fixed speed of 18 meters/min with and an inclination of 5.0% grade. Running exercise was performed in non-fasted animals at the same time of the day at 12:00 am throughout the study. To minimize the psychological stress that might interfere with exercise performance or disguise exercise familiarizations, before the initiation of exercise bout, mice were adapted to the treadmill five min-rest and five min-running at 5 meter/min and zero inclination each day for 3 days (Mahmud and Al-Domi, 2014).

Given that mice are nocturnal, treadmill familiarization and training were conducted during their dark cycles (active period). In order to control for stress that might be associated with the exercise protocol, mice in the C-Sal-Sed group were exposed to the same noise and handling as those in the exercise group. A 100 µl of exendin-4 (Lot no. E7144-100, Sigma, St. Louis, MO) was administered intraperitoneally (IP) at a dose of 1 nmol/kg body weight once a day to all mice in the exendin-4 groups. Control groups were treated with a volume matched saline. Exendin-4 or saline was injected at 11:00 am every day for 13 weeks (Mahmud and Al-Domi, 2014).

**Body mass index, caloric intake and feed efficiency:** Individual body weight and length were measured twice a week throughout the study. BMI was calculated by dividing body weight in grams (gm) by the square of the mouse length in centimeters squared (cm<sup>2</sup>). Water and food intakes were measured daily. Energy (Kcal) intake was calculated by multiplying the weight of food consumed (gm) by the number of kilocalories per gram (kcal/gm) of the food for each day. Feed efficiency (FE) was determined as the total weight gain in body weight of the mice divided by energy intake (Kcal) (Zigman *et al.*, 2005; Engelbregt *et al.*, 2001).

**Intraperitoneal glucose tolerance test (IPGTT):** Overnight-fasted conscious mice (16-18h) were challenged IP with 20% glucose in sterile saline (0.9%

NaCl) at a dose of 2g/kg body weight. Blood glucose levels were measured via tail bleeds at t = 15, t = 30, t = 60, t = 90 and t = 120 min after glucose administration using a glucometer (ACCU-CHEK GO, Roche Diagnostics GmbH, Germany). IPGTT was performed at the endpoint of the study (week 26) after exercise and/or exendin-4 interventions. Area under the curve for glucose ( $AUC_{\text{glucose}}$ ) was calculated for glucose from 0 to 120 min.

**Measurements lipid profile and GLP-1 levels:** At week 26, all experimental mice were killed under deep anesthesia with diethyl ether. Following 2 h of their final morning exendin-4 injection and 24 h after the last exercise bout, a 250  $\mu$ l blood sample was collected from mice through retro-orbital bleeding (Zigman *et al.*, 2005). GLP-1 samples were analyzed using commercial enzyme-linked immunosorbent assay (ELISA) kit (GLP-1 active ELISA kit, Linco Research, Lot no. 1720348, 2010, USA). Estimated total cholesterol (T-Chol), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL)-cholesterol levels were measured by using bioassay systems (EnzyChrom™ AF HDL and LDL/VLDL Assay Kit, Lot no. 100331, 2010, USA). Plasma triglycerides (TG) levels were determined by an enzymatic colorimetric method using commercially available kits (Globe Diagnostics, Italy, 2010) on a semi-automated clinical chemistry analyzer (Clima Plus 707, RA133000, Spain). All assays were performed according to manufacturer's instructions and parameters were measured in duplicate.

**Statistical analysis:** Statistical analysis was performed using the SPSS for windows 2008 version 17.0. Differences between groups were examined using either Mann-Whitney test or one-way ANOVA followed by Tukey post hoc test when a significant F ratio was attained. The area under the curve (AUC) was determined by the trapezoid rule for glucose curves. Data were presented as mean  $\pm$  standard error of means. All p-values less than or equal to 0.05 were considered significant.

## RESULTS

**Effect of exendin-4 and/or exercise on weight status:** Figure 1 shows that sustained exposure of mice fed the HSFD for 26 weeks to peripheral administration of exendin-4 and/or five days a week to exercise regimen for 13 weeks resulted in a marked ( $p < 0.001$ ) reduction in the gained body weight as compared to that in the C-Sal-Sed group, consequently the BMI of mice in all groups was significantly lower than that in the C-Sal-Sed group ( $p < 0.001$ ).

**Effect of exendin-4 and/or exercise on caloric intake:** Figure 2 illustrates that mice in all groups were significantly less feed efficient than that in the control

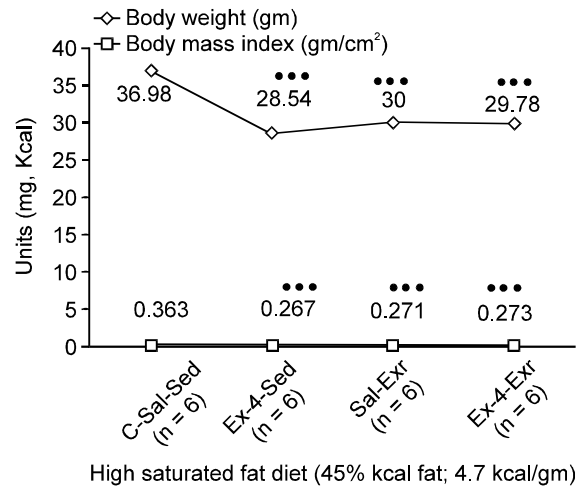


Fig. 1: Effect of 13-week of exercise and/or exendin-4 treatment on changes in body weight and body mass index in female C57BL/6 mice fed the high saturated fat diet (45% kcal fat; 4.7 Kcal/gm) for 26 weeks. Data are presented as mean  $\pm$  standard error of mean and are significant at  $**p < 0.01$ ,  $***p < 0.001$  versus control-saline-sedentary subgroup. Body mass index, calculated by dividing body weight in grams (gm) by the mouse length in centimeters squared ( $\text{cm}^2$ ); C: Control, Sal: Saline, Ex-4: Exendin-4, Sed: Sedentary, Exr: Exercise

group ( $p < 0.001$ ). There was no significant difference between the Sal-Exr group and the C-Sal-Sed group with regard to accumulative caloric intake ( $1092.37 \pm 17.38$ ,  $1020.51 \pm 27.8$ ; respectively,  $p > 0.05$ ), whereas there was significant difference between accumulative food intake and accumulative fat intake as compared to the C-Sal-Sed group ( $p < 0.01$ ), the previous indicators were significantly lower in the Ex-4-Sed and Ex-4-Exr groups than that in the C-Sal-Sed group.

**Effect of exendin-4 and/or exercise on glucose tolerance:** Figure 3 shows a significant deterioration in glucose tolerance in the control group, which was significantly improved in the mice received exendin-4 ( $p < 0.001$ ). Similarly, exercise and/or exendin-4 for 13 weeks resulted in a significant decrement in  $AUC_{\text{glucose}}$  ( $19,155.0 \pm 305$ ,  $16,852.5 \pm 725$ ,  $p < 0.001$ ) as compared to that in the C-Sal-Sed group. Overall, both  $AUC_{\text{glucose}}$  and blood glucose levels were significantly ( $p < 0.001$ ) decreased in all groups as compared to that in control group.

**Effect of exendin-4 and/or exercise on blood parameters:** Figure 4 clearly shows that glucose and TG levels were significantly lower ( $p < 0.001$ ) in all experimental groups than that in the control group.

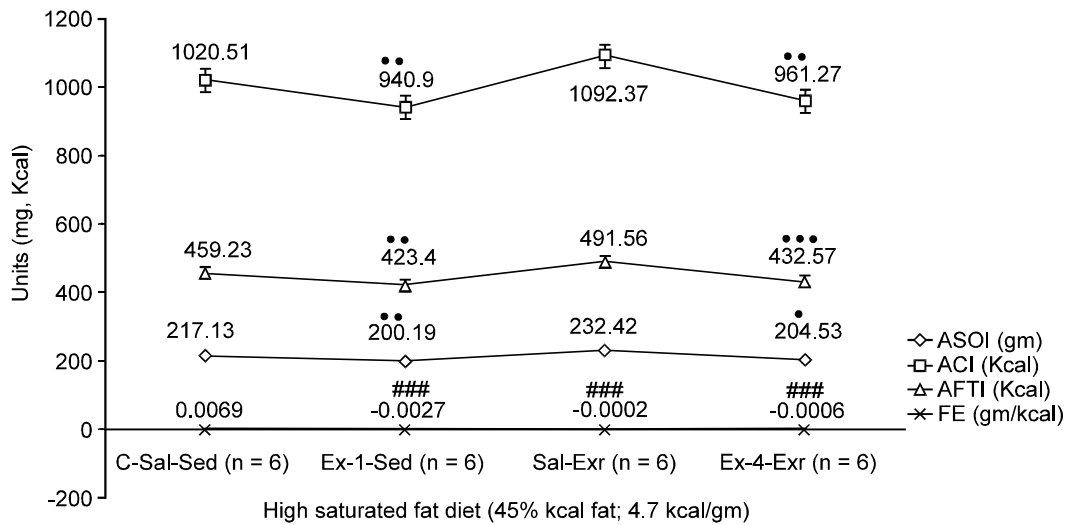


Fig. 2: Physiologic parameters in C57BL/6J female mice underwent exercise and/or exendin-4 treatment for 13 weeks and fed high saturated fat diet (45% kcal fat; 4.7 Kcal/gm) for 26 weeks. Data are presented as mean±standard error of mean and are at ###p<0.001 versus control-saline-sedentary subgroup and are significant at \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 versus Saline-exercise subgroup. AFOI: Accumulative Food Intake (gm); ACI: Accumulative Caloric Intake (Kcal); AFTI: Accumulative Fat Intake (Kcal); FE: Feed Efficiency; calculated by the total weight gain in body mass of experimental animals divided by energy intake (Kcal); C: Control, Sal: Saline, Ex-4: Exendin-4, Sed: Sedentary, Exr: Exercise

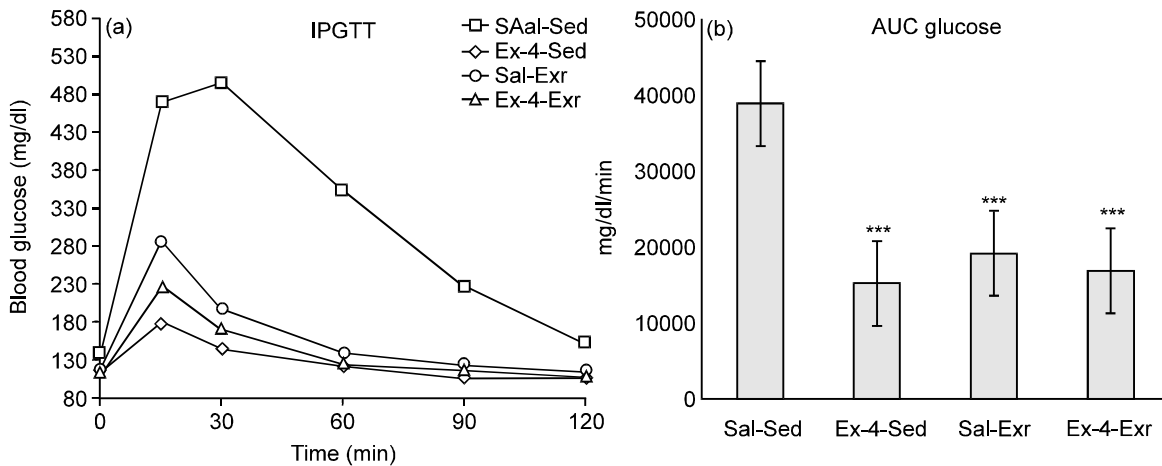


Fig. 3: Effect of 12-week treatment with exendin-4 and/or exercise on glycemic variation in female C57BL/6 mice. (A) Blood glucose levels in mice during intraperitoneal glucose tolerance test performed at the age of 30 weeks (n = 6/group). (B) Area under the curve for glucose (AUC<sub>glucose</sub>) was calculated for glucose from 0 to 120 min. Data is presented as mean±standard error of mean and are significant at \*\*\*p<0.001 versus Sal-Sed (control) subgroup. IPGTT: Intraperitoneal glucose tolerance test; Sal: Saline, Ex-4: Exendin-4, Sed: Sedentary, Exr: Exercise

Although there was no significant (p>0.05) difference in HDL levels in all groups as compared to the C-Sal-Sed group, the levels were increased in the experimental groups as compared to that in the C-Sal-Sed group. While there was no significant difference (p>0.05) in T-Chol levels in Sal-Exr and Ex-4-Exr groups, it was significantly lower in the Ex-4-Sed group than that in the

C-Sal-Sed group (p<0.01). GLP-1 levels were significantly higher in all experimental groups than that in the C-Sal-Sed group (p<0.001).

### DISCUSSION

Feeding of high fat diet has adverse effects including increase in the body weight and adiposity (Thounaojam

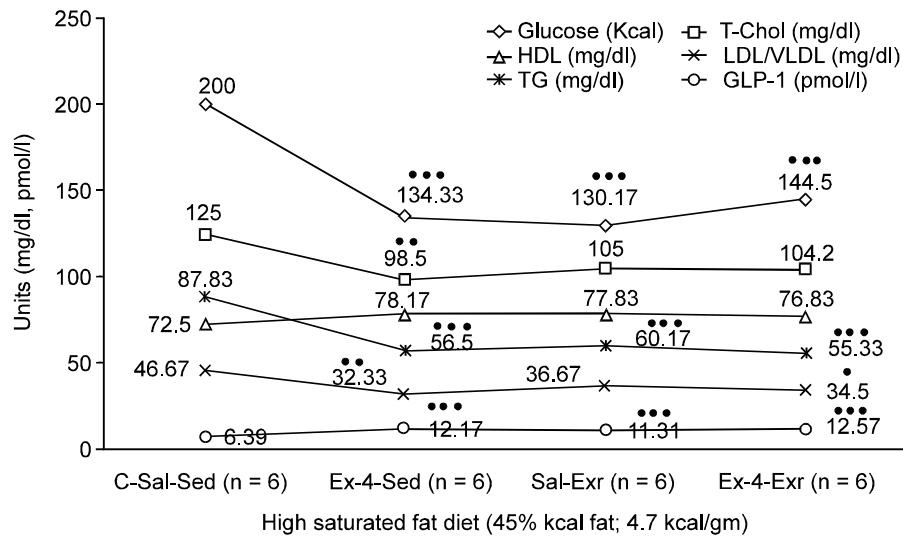


Fig. 4: Effect of exercise and/or exendin-4 treatments on some metabolic parameters in C57BL/6 female mice underwent exercise and/or exendin-4 treatment for 13 weeks and fed the high saturated fat diet for 26 weeks and. Data are presented as mean±standard error of mean and are significant at \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, 001 versus control-saline-sedentary subgroup. T-Chol: Total cholesterol; HDL: High density lipoprotein; LDL/VLDL: Low density lipoprotein / Very low density lipoprotein; TG: Triglyceride; GLP-1: Glucagon-like peptide-1. All parameters were determined in the fed state. C: Control, Sal: Saline, Ex-4: Exendin-4, Sed: Sedentary, Exr: Exercise

et al., 2010). Earlier findings of Mahmud and Al-Domi (2014) demonstrated that short term (13 weeks) feeding of high-fat-diets induced-obesity in pathogen-free female C57BL/6 mice. Similarly, long-term (26 weeks) feeding of the high fat diets without exercise resulted in further obesity induction and also resulted in hyperglycemia, hypertriglyceridemia and hypercholesterolemia. Moderate intensity exercise performed in the fed-state caused a significant reduction in both body weight and BMI. While exercise was not able to affect total energy intake or to alter TC and HDL levels, it was able to normalize impaired glucose tolerance caused by high fat diets and sedentary state. In addition, weight loss induced by long-term exercise intervention significantly increased intact GLP-1 levels (p<0.01) as compared to the sedentary mice without the need for changing the fat content of the diet (Mahmud and Al-Domi, 2014).

The findings of the current study showed that mice fed the HSFd in exendin-4 and/or exercise groups were significantly less feed efficient than that in the C-Sal-Sed group, which are in agreement with our earlier study (Mahmud and Al-Domi, 2014). This could signify that mice efficiently converted diet energy into body mass buildup and consequently accumulation of body fat. Administration of exendin-4, a GLP-1 receptor agonist, induced weight loss and inhibited food intake (Szayna et al., 2000). Moreover, our findings showed that cumulative food intake did not demonstrate significant anorectic effects in mice fed the HSFd and subjected to 13 weeks exendin-4 treatment as compared to C-Sal-Sed group.

Greig and colleagues found that short-term (one week) injection of exendin-4 to db/db mice has short-lived anorectic influence; by the end of the experiment, food intake does not differ from that in vehicle-treated mice; as such, exendin-4 may act as a taste aversive agent than being anorectic agent (Greig et al., 1999). On the other hand, Reidelberger and colleagues suggested that the declining anorectic effect of exendin-4 across the 10-week period treatment with diet induced-obesity in rats is likely due to receptor downregulation and tolerance to repeated or high doses of exendin-4 (Reidelberger et al., 2011).

Interestingly, even greater importance of exendin-4 treatment, we demonstrated that despite the loss of the anorectic action of exendin-4 in mice fed the HSFd, no compensatory increase in food intake was observed throughout the 13 weeks of treatment of exendin-4. This was inconsistent with the findings of Szayna et al. (2000) who demonstrated that although 14 days of once daily (10 µg/kg) treatment with exendin-4 on food intake was short lived in Zucker (fa/fa) rats kept on a chow diet, no compensatory increase in food intake over than that in the control group. Although the mechanism by which exendin-4 might affect hypothalamic neuropeptide expression remains unknown yet, the absence of a compensatory effect on weeks following the repeated daily administrations of exendin-4 potentially could exhibit the downregulation of orexigenic signaling within the hypothalamus (Scott and Moran, 2007).

We demonstrated that treatment with exendin-4 for 13 weeks resulted in a persistent reduction in body weight and BMI ( $p < 0.001$ ). Similarly, Szayna *et al.* (2000) showed that daily injection with exendin-4 caused a significant reduction in body weight and concomitant reduction in visceral fat storage. The mechanism underlying the progressive reduction in body weight and adiposity with no detectable changes in food or caloric intake in mice treated with exendin-4 and fed the HSFd could be ascribed to the increase in energy expenditure (Samson *et al.*, 2008).

We demonstrated that daily administration of exendin-4 for 13 weeks to obese glucose intolerant mice induced by the HSFd was accompanied by a reduction in post-meal hypertriglyceridemia, hyperglycemia and hypercholesterolemia as compared to the elevated levels of TG, T-Chol and glucose among the control group ( $p < 0.05$ ). We also showed that exendin-4 invoked marked suppression in serum LDL/VLDL levels; similar findings were reported by Mack and colleagues who demonstrated that exendin-4 induced substantial weight loss, thus exendin-4 exerts a concomitant improvement on the metabolic profile (Mack *et al.*, 2006). Repeated administration of exendin-4 has long-term glucose-lowering effect in diabetic mice (Greig *et al.*, 1999). The mechanism underlying the glucose-lowering effect of exendin-4 in the fed-state can mainly be explained by decreasing gastric emptying and secretion, which, in turn, delays the absorption of ingested nutrients and therefore contributes to the subsequent reduction in meal-associated increase in glycemic variation (Chia and Egan, 2005). In addition, the suppression of glucagon release and insulinotropic effects might be another contributing factor (Kolterman *et al.*, 2003). Interestingly, our findings demonstrate that levels of the intact GLP-1 are significantly higher in mice treated daily with exendin-4 than that in the control group ( $p < 0.01$ ). This can be explained by the ability of exendin-4 to retain native GLP-1 properties and demonstrate long acting characteristic; there by serving as a potent GLP-1 receptor agonist.

Our earlier findings demonstrated that moderate intensity exercise was associated with substantial improvement of several metabolic and physiologic parameters in C57BL/6 female mice and resulted in remarkable weight reduction ( $p < 0.001$ ) and feed efficiency and concomitant normalization of glucose intolerance ( $p < 0.01$ ), significant increment of the plasma GLP-1 levels ( $p < 0.01$ ), as well as the apparent rise in TG levels caused by long-term intake of the high fat diets were retained via exercise ( $p < 0.01$ ) (Mahmud and Al-Domi, 2014). The apparent difference in GLP-1 levels enhanced by exercise might be attributed to a remarkable weight loss, thereby implying a potential reverse of the decremental influence of obesity (Mahmud and Al-Domi, 2014).

Moreover, the finding of the present study demonstrated that exercise was capable of inducing a remarkable decrease in TG levels. Positive effect of exercise training after long-term fat diet adaptation on dyslipidemia may be attributed to fat utilization via enhancing lipid oxidation during exercise. Exercise in obese individuals could induce the breakdown of TG and postprandial hyperlipidemia through stimulating lipoprotein lipase activity during low-intensity exercise (Heim *et al.*, 2000). In addition, except for LDL/VLDL levels, exercise showed no effect on LDL/VLDL, HDL and T-cholesterol levels ( $p > 0.05$ ) even when exercise was accompanied by exendin-4; exercise stimulus could be insufficient and a higher intensity exercise is required to correct dyslipidemia observed in obese mice fed-high fat diets. We also demonstrated that hyperglycemia was significantly decreased and deterioration in glucose tolerance was significantly improved following 13-week of exercise, which are in agreement with the findings of Park and colleagues (Park *et al.*, 2008). The substantial influence of exercise on improving hyperglycemia potentially reflects the enhancement in glucose uptake in skeletal muscles and adipose tissues (Barrera *et al.*, 2009). Furthermore, enhancement in pancreatic beta-cell function and mass exerted by regular exercise is likely ascribed to the insulinotropic effect equivalent to exendin-4 through a common pathway of exendin-4 in rats with type 2 diabetes and insulin resistance (Park *et al.*, 2008).

**Conclusion:** In conclusion, this study provides evidence that peripheral treatment with exendin-4 alone and/or moderate intensity of exercise is effective interventions that invoked sustainable antiobesity effect reflected by a significant reduction in both body weight and adiposity and concomitant improvement in some pathophysiological abnormalities associated with obesity resulting from long-term feeding of HSFd; these including enhancement of GLP-1 levels in C57BL/6 obese mice. This provides new insights into the importance of applying specific clinical intervention methods to prevent or at least to ameliorate the occurrence of obesity.

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