Reversal of Obesity and Metabolic Disorders by Exercise in High-Fat Diet-Induced Obese C57BL/6 Mice

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ABSTRACT

Obesity has become a major global health challenge. The rising prevalence of overweight and obesity in several countries has been described as a global pandemic. The objective of this study was to examine whether 13 weeks of moderate aerobic exercise performed in the fed-state could reverse metabolic disorders associated with obesity in a C57BL/6 female mice fed diets high in saturated or unsaturated fats. A total of 31 female C57BL/6 five weeks old mice were randomly assigned to three groups: a normal fat diet (NFD) group, a high saturated fat diet group (HSFD), or a high unsaturated fat diet group (HUSFD). Mice were fed their respective diets for 13 weeks after which exercise regimen was undertaken. Mice fed the high fat diets were further subdivided into sedentary and exercise groups (13 weeks exercise). Mice fed the NFD continued on the same diet without exercise treatment. Glucose, lipid profile, and glucagon-like peptide-1 (GLP-1) levels were analyzed after 13 weeks of diet induction, and at the end of the study. Intraperitoneal glucose tolerance tests were carried out after 12 weeks of the diet induction, and at 25th week of the study. 13 weeks of feeding the HFD caused obesity among experimental groups as compared to NFD group which maintained normal body weight. Glucose levels were significantly higher in the fed the high fat diet (HFD) groups (168.6±3.2 and 167.25±2.2) than that in the NFD group (123.86±2.9, P<0.001). Similarly, TC, TG and HDL levels were significantly higher in the HFD groups than that in the controls; whereas, GLP-1 levels were significantly reduced in the HSFD and HUSFD groups as compared to the controls (6.46±0.3, 7.97±1.2, and 10.56±0.3; respectively, *P*<0.001). GLP-1 levels were significantly higher in the HUSFD than that in the NFD group (P<0.001). Mice in the groups fed the HFD gained significantly more body weight than that in the NFD group and became glucose intolerant (*P*<0.001). Exercise for 13 weeks resulted in decreased both hypertriglyceridemia (*P*<0.01) and hyperglycemia (*P*<0.001) observed in the sedentary groups. It also significantly reduced body mass index (*P*<0.001) and feed efficiency (*P*<0.05). While GLP-1 levels were significantly lower in the sedentary HFD groups than that in the NFD group, GLP-1 levels in the HFD exercise subgroups were significantly higher than that in the NFD group. Glucose intolerance was improved in the HFD exercise subgroups as compared to the sedentary counterparts and the NFD group. Exercise had no effect (*P*>0.05) on serum levels of total cholesterol and high density lipoprotein in mice fed the HFD. In conclusion, exercise reverses obesity and certain metabolic disorders resulting from long-term feeding of HFD regardless of the need for dietary modification; these beneficial effects of exercise could be mediated by a significant increase in GLP-1 levels following weight loss. This study therefore may support that exercise is an effective treatment for obesity.

Keywords: Exercise, high-fat diet, obesity, glucagon-like peptide-1.

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1. INTRODUCTION

Obesity has emerged as a major global health challenge. The substantial rise in its prevalence of both overweight and obesity in developed and developing countries has been described as a global pandemic (WHO, 2011). In Jordan, more than 38% of females and 20% of males are obese (Badran and Laher, 2011). The obesity global pandemic is a result of multifactorial complex interplay between genetic predisposition, and dietary and non-dietary environmental risk factors (Yang *et al*. 2007).

In the face of the rising prevalence of overweight and obesity, physical exercise is one of the first-line treatments in weight management and glycemic control (Adam and Westertrep-Plantenga, 2004). It has been widely accepted that physical activity over the past few decades has decreased due to changing lifestyle (Martins *et al.,* 2008). The most recent Physical Activity Guidelines for Americans suggested that for substantial health benefits, adults should perform at least 150 minutes per week of moderate-intensity exercise (U.S. Department of Health and Human Service, 2010). Exercise in the fed-state could exert its beneficial effect on the maintenance of body weight through controlling appetite. This is likely through providing alterations in the release of satiety hormones produced from the gastrointestinal tract (GI), which are implicated in the short-term appetite control (Martins *et al.,* 2008).

Obesity and diabetes mellitus are associated with a decline in glucagon-like peptide-1 (GLP-1) levels (Baggio and Drucker, 2007). GLP-1 is one of the known satiety hormone released from the intestinal endocrine cells following food ingestion, primarily from carbohydrates and fats (Holst, 2007; Chia and Egan, 2005). GLP-1 exerts a spectrum of actions, including promoting glucose homeostasis, inhibiting gastrointestinal secretion and motility, and notably gastric emptying (Girard, 2008; Larsen and Holst, 2005). This could play a principal role in the postprandial glucose regulation (Barnett, 2009; Girard, 2008) and the decrease of food consumption (Neumiller, 2009; Verspohl, 2009) as well as improving postprandial lipemia (Meier *et al.,* 2006).

Exercise is one of the most prescribed measures to prevent weight gain (Marttinez-Gonzalez *et al*., 1999; Haapanen *et al*., 1997), yet its ability to favorably reverse cardiometabolic disorders associated with obesity remains controversial, especially in the absence of energy restriction. Hence, the objective of this study was to examine whether moderate aerobic exercise while in the fed-state could reverse certain metabolic disorders associated with obesity in female C57BL/6 mice fed high saturated or unsaturated fat diets.

2. MATERIALS AND METHODS

2.1. Experimental Animals and Diets

A total of 31 inbred pathogen-free female C57BL/6 mice (Varela-Rey, 2009) with an average body weight of 14.5 gm were purchased from Harlan Laboratories (Canada, 2010) through the Committee of the Product Safety Research Laboratories-Royal Scientific Society (PSRL-RSS), Amman, Jordan at 4-5 weeks of age. Animals were maintained in the Pharmaceutical Research Unit-Royal Scientific Society (PRU-RSS), Amman, Jordan, and were housed individually in a specific pathogen-free facility under an automatic reverse lighting schedule (lights on at 23:30 and off at 11:30) with a constant temperature of 23±2˚C and relative humidity of 50±10%. All experiments were carried out in conformity with the recommendations of the guide for the care and use of laboratory animals (The National Academy of Sciences, 1996), and were approved by the Institutional Animal Care and Use Committee of the PSRL-RSS.

Mice were acclimatized to the animal facility in the PRU-RSS without handling for one week during which

animals were fed *ad libitum* standard chow diet (Rodent NIH-07; 29% energy derived from protein, 15% from fat, and 56% from carbohydrates, PSRL-RSS, Amman, Jordan, 2010). Tap water was supplied freely using water bottles. Mice were then randomly allocated to three diet groups: (group 1) high saturated fat diet (HSFD, *n*=12, 4.7 Kcal/g, 45 Kcal % fat); (group 2) high unsaturated fat diet (HUSFD, *n*=12, 4.7 Kcal/g, 45 Kcal % fat); and (group 3) normal fat diet (NFD-control, *n*=7, 3.8 Kcal/g, 10 Kcal % fat).

Mice were fed the HSFD, HUSFD, and NFD for 13 weeks prior to the initiation of exercise regimen; HSFD and HUSFD were provided to mice to induce obesity. All experimental diets were prepared by Research Diets, Inc. (Lot. no. 10021107A8, 2010, USA). The compositions of the experimental diets are shown in Table 1.

2.2. Experimental Protocol

Following the 13 weeks fattening period, mice fed the high-fat-diets (HFD) were further subdivided randomly into either sedentary or exercise subgroups (6 mice/group). Mice in the control group continued on NFD until the completion of the study without exercise treatment. A moderate intensity exercise regimen 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 an inclination of 5.0% grade. Running exercise was performed in non-fasted animals throughout the study (Davis *et al*., 1997).

Intraperitoneal glucose tolerance tests were undertaken at baseline (prior to the exercise treatment) and at one week before the end of the study (week 25). Capillary whole blood was drawn to determine glucose levels by using glucometer. Mice were weighed to measure the amount of blood that can be safely withdrawn from each mouse to avoid mice shock. A 250 µL blood sample was withdrawn from each mouse through retro-orbital bleeding at baseline prior to the exercise treatment, and at the endpoint of the study (week 25). At week 26, all mice were sacrificed under deep anesthesia with diethyl ether. All experimental mice accomplished the exercise regimen successfully (Zabalawi *et al.,* 2003; Furbee *et al.,* 2001).

Individual body weight and length were measured weekly over the 26 week period. Body mass index (BMI) was calculated by dividing body weight in grams (gm) by the square of the mouse length in centimeters squared (cm^2) (Engelbregt *et al*., 2001). Mice were classified as normal weight if their BMI was 0.18 to 0.25 gm/cm^2, overweight if BMI >25-30, or obese if BMI ≥ 30 gm/cm^2 (Hong *et al*., 2009). Length of the mouse was measured from the tip of the nose to the base of the tail using a linear ruler (Haskell-Luevano *et al*., 2009; De Bono *et al.,* 2006). Daily food intake was measured by subtracting the weight of food spillage from the initial weight of food provided using a balance with a precision of ±0.01 gm (Startorius, GMBH Germany).

Food intake was measured over a 24-h period for twenty-six weeks; results were expressed as grams of food intake per day. Energy intake was calculated by multiplying the weight of food consumed (in grams) by the number of kilocalories per gram (kcal/gram) of the food for each day. Feed efficiency was determined as the total weight gain in body weight of the experimental animals divided by energy intake (Kcal). Metabolic efficiency was calculated as energy intake divided by body weight gain (Thounaojam *et al.,* 2010).

Plasma GLP-1 (200 µL) levels were analyzed using commercial enzyme-linked immunosorbent assay (ELISA) kit (GLP-1 active ELISA kit, Linco Research, Lot no. 1720348, 2010, USA) specific for measurement of biologically active (intact) GLP-1 (7-36) amide without cross reactivity towards other forms of GLP-1; GLP-1(9-36) amide, GLP-2, or glucagon. Plasma

samples were prepared by centrifugation of blood at 4˚C for 10 min at 1000xg (Sigma; Model 1-15, Germany). Plasma samples were kept frozen at -80°C until used (Mahmud and Al-Domi, 2013).

Frozen (-20˚C) serum samples (50 µL) were thawed for measuring the levels of glucose, triglyceride (TG), high density lipoprotein (HDL), and total cholesterol (TC). Glucose levels were measured using bioAssay systems (QuantiChrom^{TM} Glucose Assay Kit, Lot. no.100517, 2010, USA). TC and HDL were measured by using commercially available bioassay systems (EnzyChrom^{TM} AF HDL and LDL/VLDL Assay Kit, Lot no. 100331, 2010, USA). TG serum concentration was determined by enzymatic colorimetric method using commercially available kit (Globe Diagnostics, Italy, 2010) on a semi-automated clinical chemistry analyzer.

2.3. Statistical Analysis

The 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 (Matthews *et al.,* 1990). Data were presented as mean± standard error of means. All P values less than or equal to 0.05 were considered as significant.

3. RESULTS

3.1. Effect of 12 weeks feeding period of HFD on glucose tolerance

Figure 1A and 1B show the effect of 12 weeks feeding period of the HFD on glucose tolerance in experimental groups prior to the exercise treatment.

$AUC_{glucose}$ values following 12 weeks feeding period were significantly higher in mice fed the HSFD and HUSFD groups than that in the NFD controls (36061.5±1270.42, 31124.25±1344.9, and 23418.21±944.2 mg/dl. 120min; respectively, P<0.001). Similarly, the previous indicator was significantly (P<0.05) higher in the HSFD than that in the HUSFD.

3.2. Effect of 13 weeks feeding period of HFD on some metabolic characteristics prior to exercise.

Table 2 reveals the effect of 13 weeks feeding period of the NFD, HSFD and HUSFD on some metabolic biomarkers prior to exercise. Glucose levels were significantly higher in the HSFD and HUSFD groups than that in the NFD group (168.6±3.2 and 167.25±2.2; 123.86±2.9; respectively, P<0.05). Similarly, TC, TG and HDL levels were significantly higher in the HFD than that in the NFD control group. GLP-1 levels in the HSFD and HUSFD groups were significantly lower than that in the NFD group (6.46±0.26, 7.97± 1.18, and 10.56 ±0.35 pmol/L; respectively, P<0.05). GLP-I levels were the lowest in the HSFD group (P <0.001) as compared to that in the HUSFD. There was no significant difference between all diet groups with regard to HDL levels (P>0.05).

3.3. Effect of 12 weeks exercise regimen on glucose tolerance in mice fed HFD for 25 weeks.

Figure 2A and 2B show that long-term consumption of the HSFD and HUSFD led to significant increase in IGT quantified over the entire 120-min as compared to that in the NFD group (38850.0±851, 33295.0±1128, and 23242.5±1837 mg/dL.120min; respectively, P<0.01). Glucose impairment was significantly improved in mice subjected to exercise treatment in both the HSFD and HUSFD groups as compared to that in NFD sedentary group (19,155±305.1 and 16, 852.5±725 mg/dL.120min, 23242.5; respectively, P<0.001). $AUC_{glucose}$ values in the HUSFD group subjected to exercise treatment were significantly even less than that in the NFD group (P<0.05).

3.4. Effect of 13 weeks of exercise regimen on certain physiologic indicators in mice fed HFD for

26 weeks.

Table 3 shows that body weight of mice fed the HUSFD and HSFD for 13 weeks was significantly higher than that in NFD group (29.44±0.6, 30.35±.0.4, and 24.63±0.7; respectively, $P<0.001$). As such, mice fed the HUSFD and HSFD developed obesity as compared to NFD group (0.304±.0.01, 0.319±0.004, and 0.248±0.006; respectively, $P<0.001$). In addition, percent change in BMI of the HSFD group was significantly higher than that in HSFD group (49.54%, and 40.77%; respectively, $P<0.001$). Similarly, long-term (26 weeks) HUSFD and HSFD feeding without exercise induced obesity as compared to mice fed the NHD (BMI: 0.322±0.01 gm/cm^2, 0.363±0.01 gm/cm^2, and 0.251±0.01 gm/cm^2; respectively, $P<0.001$). Whereas, after 13 weeks of moderate exercise, there was no significant difference between the high-fat-fed mice and the NFD group HFD (*$P>0.05$)*. 13 weeks exercise treatment resulted in a significant reduction in BMI in mice compared to their counterparts in the sedentary groups ($P<0.001$).

No significant difference was observed between all groups regarding the total caloric intake ($P>0.05$). Moreover, mice underwent exercise treatment were significantly less feed efficient ($P<0.001$) in the HSFD (-0.0002±0.001) and HUSFD subgroups (-0.0017±0.001) than that in the sedentary counterpart subgroups (0.0069±0.001, and 0.0066±0.001; respectively, $P<0.001$). This was even less feed efficient than mice in the NFD group (0.0029±0.001, $P<0.05$). On the contrary, sedentary mice fed the HSFD and HUSFD were significantly higher feed efficient than that mice fed the NFD ($P<0.01$ and $P<0.05$; respectively).

3.5. Effect of 13 weeks of exercise on some metabolic biomarkers in mice fed HFD for 26 weeks.

Table 4 shows the effect of exercise regimen on certain metabolic biomarkers at the endpoint of the study (week 26). Exercise treatment for 13 weeks was able to significantly ($P<0.01$) decrease both hyperglycemia and hypertriglyceridemia in mice fed the HSFD and HUSFD for 26 weeks as compared to their counterpart sedentary mice fed either the HFD or the NFD. There was significant increase in GLP-1 levels in both HSFD and HUSFD groups as compared to that in sedentary subgroups (11.31±0.5, 15.43±0.3, 6.39±0.4, and 9.03±0.4; respectively, $P<0.01$). Although there was no significant ($P>0.05$) difference between groups, exercise decreased TC levels and increased HDL levels in mice fed HSFD and HUSFD as compared to the mice fed NFD for 26 weeks.

4. DISCUSSION

Findings of our study are fivefold. Firstly, 13 weeks of feeding the HFD induced obesity among experimental groups. Secondly, moderate intensity exercise for 13 weeks by treadmill running performed in the fed-state animals resulted in significant reduction in BW and BMI ($P<0.001$). Thirdly, exercise normalized IGT and retained the accumulation of serum TG caused by HFD and sedentariness ($P<0.01$). Fourthly, weigh loss induced by long-term exercise intervention significantly increased intact GLP-1 levels ($P<0.01$) as compared to sedentary groups without the need for changing the fat content of the diet. Finally, exercise did not affect total energy intake; it also failed to alter TC and HDL levels.

We found that short term (13 weeks) feeding of HFD significantly induced obesity in the HSFD and HUSFD as compared to the lean NFD *(*$P<0.001$). As such, long-term (26 weeks) feeding of the HFD without exercise resulted in further obesity induction and also resulted in hyperglycemia, hypertriglyceridemia, and hypercholesterolemia compared to that in sedentary mice fed the NFD ($P<0.01$). This is consistent with previous findings wherein dyslipidemia was clearly observed in

C57BL/6 mice fed the HFD for 16 weeks or HSFD for 34 days compared to the NFD group (Thounaojam *et al.,* 2010; Libinaki *et al*. 1999).

The findings of the current study demonstrated that unlike mice subjected to 13 weeks moderate exercise, mice fed either the HSFD or HUSFD for 13 and 26 weeks without developed mild and severe glucose intolerance; respectively as compared to mice fed the NFD and also exhibited dyslipidemia. These metabolic abnormalities could be attributed to long-term fat intake without exercise, which could be correlated with insulin resistance and IGT in humans (Kibenge and Chan, 2002). Hence, lipid abnormalities associated with hyperglycemia could be used as an indicator of early manifestation of IGT (Laakso and Barrett-Connor, 1989).

Insulin resistance usually predisposes the development of IGT and type 2 diabetes mellitus (Skarfor *et al*., 1991). Increased glucose response at 30 min during the IPGTT observed in our study reflects impaired first-phase insulin release (Gerich, 2002). The abolition of first-phase insulin secretion has been found not only in overt type 2 diabetes mellitus, but also at the initial stage of the disease (i.e. IGT and impaired fasting glucose) (Guillausseau *et al*., 2008).

The postprandial hypertriglyceridemia observed in mice fed the HFD could be an indicator of poor blood TG clearance which is often associated with obesity (Gotto, 1998). Fat gained in C57BL/6 mice has been reported to deposit selectively in the mesentery (Reuter, 2007). Altered metabolism of TG-rich lipoproteins is crucial in the pathophysiology of dyslipidemia associated with insulin resistance among individuals with IGT and type 2 diabetes (Miccoli *et al.,* 2001; Laakso and Barrett-Connor, 1989).

Sensible evidence suggests that obese individuals have abnormal fasting and postprandial gastrointestinal hormones compared to normal weight individuals (Austin, 2009). The findings of the present study demonstrated that GLP-1 levels were markedly decreased ($P<0.001$) in mice fed either the HSFD or HUSFD as compared to the NFD group with the lowest levels observed in HSFD-fed mice ($P<0.05$). This finding is consistent with a study that demonstrated that the consumption of the HFD for a long period of time leads to obesity and progressive decline in circulating GLP-1 levels. Compared to a high saturated fat diet, diets rich in monounsaturated fatty acids enhance GLP-1 secretion and therefore have beneficial effect on lipid status and glycemic control (Choudhary, 2004; Rocca *et al*., 2001; Thomsen *et al.,* 1999). This was not supported by Tsunoda and colleagues (1998) who demonstrated that *ad-libitum* consumption of diet high in monounsaturated fatty acids by C57BL/6 mice for 17 weeks resulted in obesity and hyperglycemia. These findings are consistent with our study suggesting that even a diet high in monounsaturated fat when given in large quantity predisposes to obesity and diabetes. The precise mechanisms that relate GLP-1 secretion to obesity are not fully understood, yet. Part of the effect of obesity on GLP-1 secretion could be attributed to insulin resistance and IGT (Knop *et al.,* 2008).

Exercise is one of the most common recommended remedies for obese persons to lose and to maintain weight loss (U.S. Department of Health and Human Service, 2010; Marttinez-Gonzalez *et al.,* 1999; Haapanen *et al*., 1997) as well as to improve metabolic profile (Lee *et al*., 2006; Martins and Redgrave, 2004; Gandapur *et al*., 2001). Twelve weeks of exercise in C57BL/6 mice fed the HFD that provides 35% of total energy resulted in decreased body weight (Lee *et al*., 2006), which is consistent with the findings of our study. The progressive decline in BW and BMI without detectable changes in caloric intake in mice fed the HSFD could be, in part, attributed to increased energy expenditure.

Energy expenditure could be affected by exercise. We suggest that energy expenditure could be induced by GLP-1, which was significantly increased in response to exercise treatment. GLP-1 could increase energy expenditure when administered centrally and peripherally in rats, thereby supporting that GLP-1 may acts as a negative regulator of energy balance (Samson *et al*., 2008; Osaka *et al.,* 2005; Hwa *et al.,* 1998).

One of the mechanisms that may explain the positive effects of exercise training on lipid profile after a long-term fat diet adaptation demonstrated in our study could be ascribed to fat utilization by increased lipid oxidation during exercise (Marques *et al*. 2010). Increased GLP-1 levels induced by exercise could suppress the post-meal increase in triglyceride levels in individuals with type 2 diabetes, which might improve postprandial lipemia. The suppressive effect of GLP-1 on postprandial lipemia could be attributed to delay in gastric emptying and insulin-mediated inhibition of lipolysis induced by GLP-1. Furthermore, the increased clearance or reduced endogenous synthesis of triglycerides could be a contributing factor (Meier *et al.,* 2006).

Our findings demonstrated that exercise significantly decreased hyperglycemia and improved glucose intolerance (Park *et al.,* 2008). The beneficial effects of exercise could be explained by glucose-lowering actions of GLP-1, which is mediated via numerous mechanisms that include stimulation of glucose-dependent insulin release, restoration of first phase of insulin secretion, suppression of glucagon secretion and deceleration of gastric emptying (Neumiller, 2009; Verspohl, 2009). The deceleration of gastric emptying by GLP-1 and decreasing gastric secretion will, in turn, delay the absorption of ingested nutrients, thereby contributing to the subsequent reduction in meal-associated increase in glycemic variation (Barnett, 2009; Chia and Egan, 2005). On the other hand, exercise showed no effect on HDL levels (P>0.05). Discrepancies in the findings suggest that exercise stimulus was insufficient and thus a higher stimulus is required to produce changes in HDL particularly when diet is high in fat.

The present study has a few limitations. First, although glucose tolerance test provides enough information about insulin response to glucose load and healthy pancreatic islets *beta*-cells response, insulin resistance was not directly measured. Second, the control group was not subjected to exercise intervention as that in the HFD groups.

5. CONCLUSION

It can be concluded that the exercise of mice when performed in the fed-state in mice could be an effective intervention for improving glucose intolerance and elevated TG levels as well as decreased GLP-1 levels in addition to reversing obesity that result from long-term feeding of the HFD without dietary modification. The significant weight loss caused by exercise could be a crucial step in improving metabolic disorders associated with obesity.

6. ACKNOWLEDGEMENT

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Table1. Composition of the normal and high-fat-diets.

Type of diet*	**Norma fat (Control)**		**High saturated fat**		**High unsaturated fat**	
%	gm	Kcal	gm	Kcal	gm	Kcal
Protein	19	20	24	20	24	20
Carbohydrate	67	70	41	35	41	35
Fat	4	10	24	45	24	45
Total		**100**		**100**		**100**
Kcal/gm	**3.8**		**4.7**		**4.7**	
Ingredient	gm	Kcal	gm	Kcal	gm	Kcal
Casein, 80 Mesh	200	800	200	800	200	800
L-Cystine	3	12	3	12	3	12
Corn starch	315	1260	72.8	291	72.8	291
Maltodextrine10	35	140	100	400	100	400
Sucrose	350	1400	172.8	691	172.8	691
Cellulose, BW200	50	0	50	0	50	0
Soybean oil	25	225	25	225	25	225
Butter	10	90	**177.5**	**1598**	0	0
Olive oil	10	90	0	0	**177.5**	**1508**
Mineral mix S10026	10	0	10	0	10	0
Dicalcium phosphate	13	0	13	0	13	0
Calcium carbonate	5.5	0	5.5	0	5.5	0
Potassium citrate. 1H2O	16.5	0	16.5	0	16.5	0
Vitamin mix V10001	10	40	10	40	10	40
Choline bitartrate	2	0	2	0	2	0
FD&C Yellow dye #5	0.05	0	0	0	0.025	0
FD&C Red Dye #40	0	0	0.05	0	0	0
FD&C Blue dye #1	0	0	0	0	0.025	0
Total	**1055.05**	**4057**	**858.15**	**4057**	**858.15**	**4057**

*Rodent diets with 10 Kcal% fat or 45 Kcal% from (mostly) butter or olive oil. Diets formulated by Research Diets, Inc USA. February 4, 2010. Products number: normal fat diet (Lot #: D100220403), high saturated fat diet (Lot #: D100220404; high unsaturated fat diet (Lot #: D100220405).

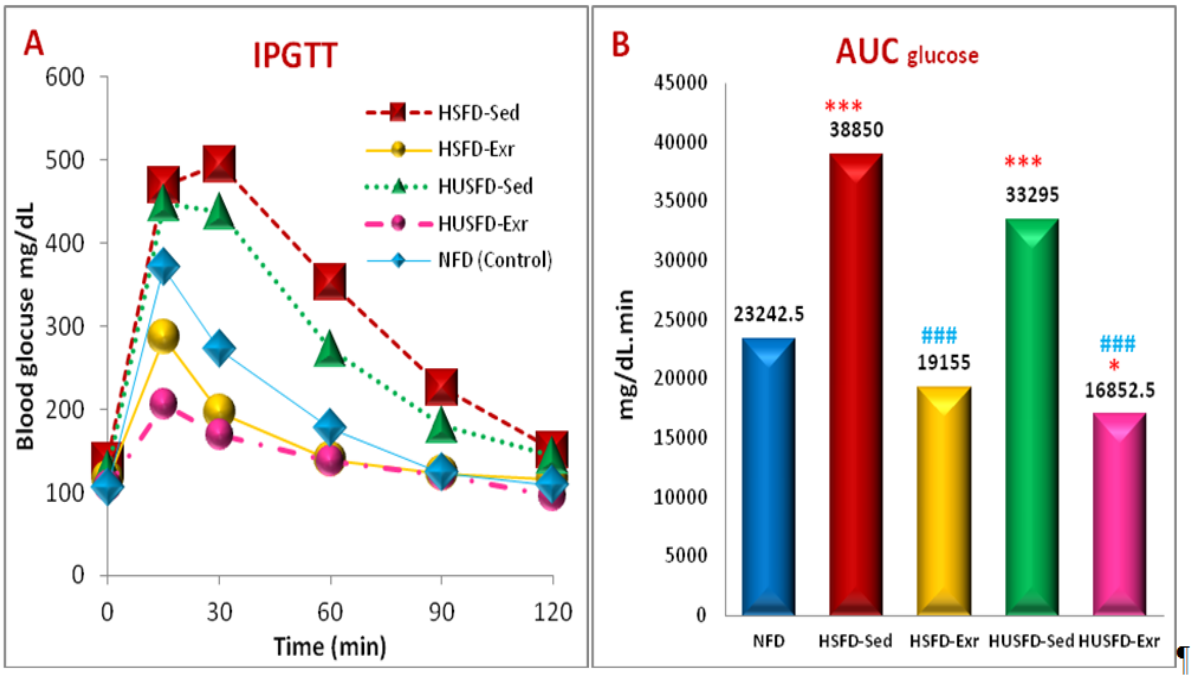

Figure1. Effect of 12 weeks of fed-high-fat-diets on glucose tolerance in mice. (A) Blood glucose concentrations measured during intraperitoneal glucose tolerance test in C57BL/6 female mice (*n*=12 per each the HSFD and HUSFD group; *n*=7/ NFD group). (B) Area under the curve for glucose ($AUC_{glucose}$) was calculated for glucose from 0 to 120 min. Data are presented as means ± standard error of mean and are significant at ***P<0.001 versus NFD group; #P<0.05 versus HSFD group. NFD: Normal fat diet (10% kcal fat; 3.8 Kcal/g); HSFD: High saturated fat diet (45% kcal fat; 4.7 Kcal/g); HUSFD: High unsaturated fat diet (10% kcal fat; 4.7 Kcal/g).

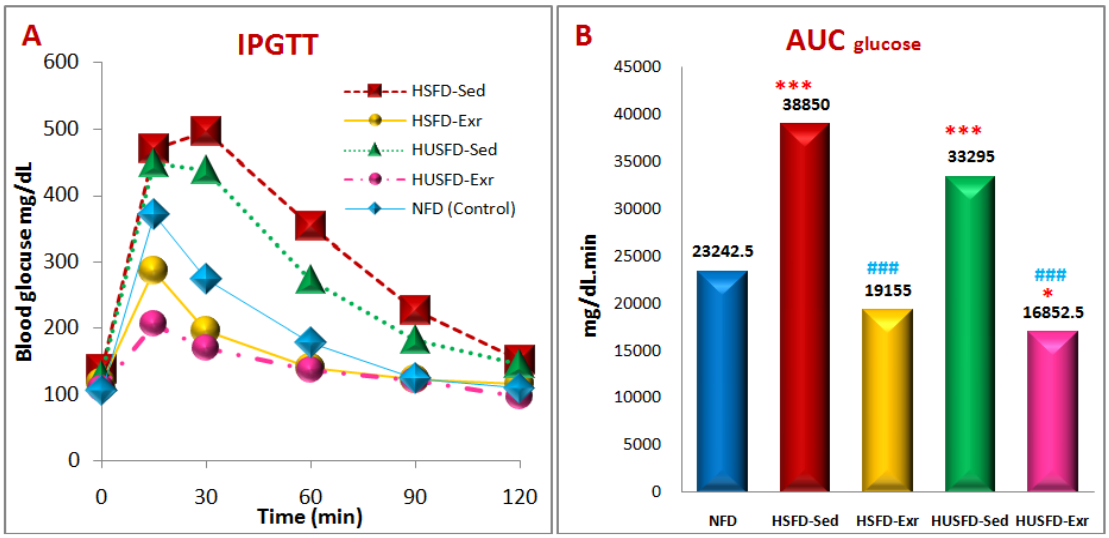

Figure 2. Effect of 12 weeks of exercise intervention on glucose tolerance in mice the fed-high-fat-diets for 25 weeks. (A) Blood glucose concentrations measured during intraperitoneal glucose tolerance test at 30 weeks (*n*=6 per each exercise subgroup fed either the HSFD or HUSFD; *n*=7/ NFD group. (B) Area under the curve for glucose ($AUC_{glucose}$) was calculated for glucose from 0 to 120 min. Data are presented as means ± standard error of mean and are significant at ***P<0.001, *P<0.05 versus NFD; ###P<0.001 versus sedentary groups. NFD: Normal fat diet (10% kcal fat; 3.8 Kcal/g); HSFD: High saturated fat diet (45% kcal fat; 4.7 Kcal/g); HUSFD: High unsaturated fat diet (10% kcal fat; 4.7 Kcal/g); Sed: Sedentary; Exr: Exercise.

Table 2. Effect of 13 weeks feeding period on high fat diets on some metabolic characteristics in mice prior to the exercise treatment.

Indicator*	Glucose† (mg/dL)	Total Cholesterol (mg/dL)	HDL (mg/dL)	Triglyceride (mg/dL)	GLP-1 (pmol/L)
NFD (*n*=7)	**123.86±2.9**	**77.43±4.2**	**63.57±3.9**	**53.29±2.9**	**10.56±0.3**
HSFD (*n*=12)	**168.67±3.2[a]**	**113.33±5.7[a]**	**72.00±3.3[a]**	**63.50±1.1[a]**	**6.46±0.3[a]**
HUSFD (*n*=12)	**167.25±2.2[a]**	**108.91±4.07[a]**	**66.08±2.9[a]**	**61.00±1.8[a]**	**7.97±1.2[a,b]**

*Values are presented as mean ± standard error of mean, and are significant at *P*<0.05 when: (a) compared to NFD, (b) compared to HSFD.
†All parameters were determined in the fed-state.
NFD: Normal fat diet (10% kcal fat; 3.8 Kcal/g); HSFD: High saturated fat diet (45% kcal fat; 4.7 Kcal/g); HUSFD: High unsaturated fat diet (10% kcal fat; 4.7 Kcal/g); GLP-1: Glucagon-like peptide-1.

Table 3. Effect of 13 weeks of exercise on certain physiologic indicators in mice fed high-fat-diets for 26 weeks.

Indicator*		Body Weight (g)		BMI (g/cm^2)	Accumulative Energy Intake (Kcal)	Feed Efficiency (gm/kcal)
		Initial†	Final‡			
NFD	Control (*n*=7)	24.63±0.7	27.50±1.03	0.251±0.01	989±23	0.0029±0.001
HSFD	Sedentary (*n*=6)	29.92±0.52[a]	36.98±0.6[a]	0.363±0.01[a]	1020.5±27.8	0.0069±0.001[a]
	Exercise (*n*=6)	30.21±1.07[a]	30.00±0.9[b]	0.271±0.01[b]	1092±17.4	-0.0002±0.001[a,b]
HUFD	Sedentary (*n*=6)	28.16±1.6[a]	34.54±0.8[a]	0.322±0.01[a]	1004±37.5	0.0066±0.001[a]
	Exercise (*n*=6)	29.23±1.06[a]	27.47±0.9[c]	0.250±0.01[c]	1026±23	-0.0017±0.001[a,c]

*Data are presented as mean ± standard error of mean, and are significant at $P<0.05$ when: (a) compared to NFD, (b) compared to HSFD-Sedentary; (c).compared to HUSFD-Sedentary.

†Body weight after 13 weeks feeding period on the HFD prior to exercise.

‡ Body weight after 26 weeks feeding HFD and 13 weeks of moderate exercise (30 min per day for five days a week).

BMI: Body mass index, calculated by dividing body weight in grams (gm) by the square of the mouse length in centimeters squared (cm2); NFD: Normal fat diet (10% kcal fat; 3.8 Kcal/g); HSFD: High saturated fat diet (45% kcal fat; 4.7 Kcal/g); HUSFD: High unsaturated fat diet (10% kcal fat; 4.7 Kcal/g).

Feed efficiency: Calculated by the total weight gain in body mass (gm) of experimental animals divided by energy intake (Kcal).

Table4. Effect of 13 weeks of exercise on some metabolic biomarkers in mice fed-high-fat diets for 26 weeks.

Indicator*		Glucose (mg/dL)†	Total cholesterol (mg/dL)	HDL (mg/dL)	Triglyceride (mg/dL)	GLP-1 (pmol/L)
NFD	**Control** (*n*=7)	142.6±3.7	81.71±4.3	65.71±3.8	59.43±3.2	10.89±0.3
HSFD	**Sedentary‡** (*n*=6)	200±5.4[a]	125.33±6.3[a]	72.5±3.8	87.83±8.8[a]	6.39±0.4[a]
	Exercise (*n*=6)	130.17±3.7[b]	105.8±5.7	77.83±3.9	60.17±1.5[b]	11.31±0.5[b]
HUSFD	**Sedentary** (*n*=6)	192.83±4.9[a]	114±3.67[a]	76.83±3.2	71.67±3.6	9.03±0.4[a]
	Exercise (*n*=6)	127.67±3.6[c]	96.17±2.6	80.83±2.5	52.67±2.3[c]	15.43±0.3[c,d]

*Data are presented as mean ± standard error of mean, and are significant at $P<0.05$ when: (a) compared to NFD, (b) compared to HSFD-Sedentary; (c).compared to HUSFD-Sedentary; (d) compared to HSFD-Exercise.

†All parameters were determined in the fed-state.

‡13 weeks of moderate exercise (30 min per day for five days a week).

NFD: Normal fat diet (10% kcal fat; 3.8 Kcal/g); HSFD: High saturated fat diet (45% kcal fat; 4.7 Kcal/g); HUSFD: High unsaturated fat diet (10% kcal fat; 4.7 Kcal/g); GLP-1: Glucagon-like peptide-1.

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ممارسة النشاط البدني يحد من السُمنة والاضطرابات الأيضية في نموذج فئران C57BL/6 يعانون من السمنة المفرطة الناجمة من تناول وجبات عالية الدهون

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ملخص

هدفت الدراسة إلى تقييم أثر النشاط البدني المعتدل في حالة الشبع لفترة 13 أسبوعا على بعض الاضطرابات الأيضية المرتبطة بالسُمنة في نموذج فئران C57BL/6 إناث سبق أن تناولت وجبات عالية في الدهون المشبعة أو غير المشبعة تم توزيع واحد وثلاثون فأرة أنثى من نوع C57BL/6 بعمر خمسة أسابيع إلى ثلاثة مجموعات إعتمادا على نوع الغذاء:(1) المجموعة الضابطة (وجبة معتدلة الدهون 10% من الطاقة دهن)، (2) مجموعة الوجبة عالية الدهون المشبعة (45% من الطاقة دهن)، و (3) مجموعة الوجبة عالية الدهون غير المشبعة (45% من الطاقة دهن). بعد 13 إسبوع من تناول الفئران لهذه الوجبات، تم إخضاع الفئران إلى النشاط البدني. بعد ذلك تم تقسيم الفئران التي تناولت الوجبات عالية الدهون إلى مجموعتين ثانويتين، (1) مجموعة خاملة بدنياً (لم تمارس نشاطاً بدنياً) و (2) مجموعة تم أخضاعها للنشاط البدني. أما فئران المجموعة الضابطة للتجربة، فقد استمرت على الوجبة معتدلة الدهون نفسها حتى نهاية الدراسة دون أن تخضع للنشاط البدني. تم قياس مستوى الجلوكوز في الدم، ، ومستوى لبيدات الدم، ومستوى الببتيد شبيه الجلوكاجون−1 بعد 13 أسبوعا من بداية إعطاء الفئران الوجبات المختلفة في محتواها من الدهون وكذلك عند نهاية الدراسة، أما إختبارات تحمل الجلوكوز داخل الصفاق فقد أجريت بعد 12 أسبوعا من بداية الوجبات وفي الأسبوع 25 من الدراسة. أدى النشاط البدني لمدى 13 أسبوعاً للمجموعات التي تناولت الوجبات عالية الدهون المشبعة وغير المشبعة إلى إلغاء الفرط الحاد ذو الدلالة المعنوية في سكر الدم ($P<0.001$)، والارتفاع الحاد ذو الدلالة المعنوية في الدهون الثلاثية ($P<0.01$)، وكذلك، فقد أدى النشاط البدني إلى انخفاض ملحوظ وذو دلالة معنوية في مؤشر كتلة الجسم ($P<0.001$)، وفي مقياس كفاءة تحويل الغذاء ($P<0.05$)، بينما أدى النشاط البدني إلى إرتفاع ملحوظ وذو دلالة معنوية في مستوى الببتيد شبيه الجلوكاجون−1 ($P<0.01$)، وتحسين ملحوظ وذو دلالة معنوية في مستوى ضعف تحمل الجلوكوز ($P<0.01$). لم يكن للنشاط البدني أي تأثير ذو دلالة معنوية على مستوى الكولستيرول في مصل الدم ومستوى البروتين الشحمي العالي الكثافة في مصل الدم ($P>0.05$)في الفئران التي تناولت الوجبات عالية الدهون المشبعة وغير المشبعة. تؤكد نتائج هذه الدراسة أن ممارسة النشاط البدني كان له تأثير في الحد من السُمنة وإضطرابات التمثيل الغذائي الناجمة عن تناول الوجبات عالية الدهون لفترة زمنية طويلة وذلك دون الحاجة إلى تعديل النظام الغذائي، وقد تكون هذه الآثار المفيدة للنشلط البدني ناجمة عن الإرتفاع الملحوظ في مستوى الببتيد شبيه الجلوكاجون−1 في بلازما الدم الناجمة عن الإنخفاض الملحوظ في الوزن . تؤيد هذه الدراسة أن ممارسة النشاط البدني يمكن أن يكون علاجاً فعّالاً بحد ذاته للحد من مرض السُمنة.

الكلمات الدالة: النشاط البدني، وجبات عالية الدهون، السُمنة، الببتيد شبيه الجلوكاجون−1.

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