Effect of Exercise and Orlistat Therapy in Rat Model of Obesity Induced with High Fat Diet

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Abstract

Background: The prevalence of obesity is increasing rapidly and, has largely been attributed to increased dietary intake of high energy food and physical inactivity. Advances in adipose tissue biology over the past decades have led to an improved understanding of the mechanisms linking obesity with obesity-associated metabolic complications. Adipose tissue is not merely a passive energy storage depot, but it is a complex, metabolically active tissue that secretes a variety of signaling molecules, collectively known as adipokines.

Material and Methods: Eight-week old male Wistar rats (n=40) weighing 280-300g divided into four groups; Group 1: Control (non obese rats): Rats which were fed standard diet (12% of calories as fat; Table 1) for 12 consecutive weeks; Group 2: HFD (obese rats): Rats which were fed high fat diet (HFD) (40% of calories as fat; Table 1) for 12 consecutive weeks; Group 3: HFD+Orlistat: Rats which were fed a high fat diet (40% of calories as fat) and received a concomitant therapeutic dose of orlistat (200mg/kg) for 12 consecutive weeks; Group 4: HFD+Exercise: Rats which were fed a high fat diet (40% of calories as fat) and underwent a simultaneous daily swimming exercise (30min/day) for 12 consecutive weeks. After 12 weeks the body weight, adipose tissue (visceral, retroperitoneal and epididymal fat) were weighed, adiposity index was calculated and lipogram, serum IL-6, TNF-a, ICAM-1, VCAM, hsCRP, ghrelin, and leptin were measured.

Results: High fat diet for 12 weeks increased significantly the body weight, adiposity index, serum total cholesterol, triglycerides LDL-cholesterol, IL-6, TNF-a, ICAM-1, VCAM, hsCRP and leptin with significant decrease in HDL-cholesterol and ghrelin as compared with control group. Both orlistat and swimming exercise decreased the body weight, adiposity index, serum total cholesterol, triglycerides, LDL-cholesterol, IL-6, TNF-a, ICAM-1, VCAM, hsCRP and leptin and significantly increased serum HDL-cholesterol and ghrelin as compared with HFD fed rats.

Conclusion: Obesity-induced low grade inflammation could be reversed by daily swimming training exercise through decreasing expression and the circulatory levels of the inflammatory mediators and cytokines.

Key Words: Exercise — Orlistat therapy — Rat model — Obesity — High fat diet.

Introduction

The prevalence of obesity continues to rise worldwide and is being accompanied by a proportional increase in the incidence of other medical conditions, such as metabolic syndrome and type 2 diabetes mellitus. Also, obesity is associated with many other disorders including: Cardiovascular diseases (CVD), chronic obstructive pulmonary diseases, breast cancer, colon cancer, dementia, and depression. Inflammation appears to be aetologically linked to the pathogenesis of all of these conditions [2-6] and the development of a chronic low-grade inflammatory state has been established as a predictor of risk for many of them. This inflammatory state is indicated by elevated levels of circulating inflammation markers, such as interleukin 6 (IL 6), tumour necrosis factor alpha (TNF-a) and C reactive protein (CRP) [7].

Importantly, physical inactivity and sedentary behaviour were reported to increase the risk of these conditions [8-10], Petersen & Pederson III. An inactive lifestyle leads to the accumulation of visceral fat, and this is accompanied by adipose tissue infiltration by pro-inflammatory immune cells, increased release of adipokines and the development of a low-grade systemic inflammatory state. In this sense, there is great interest in studying strategies to prevent or attenuate the effects of adipose tissue accumulation and the low-grade systemic inflammation associated with obesity [12]. Exercise training is considered an important environmental factor associated with body weight regulation, and this training has been shown to...
decrease chronic, low-grade systemic inflammation in humans and animals [13-15]. Thus, increasing physical activity has become an important aspect of a non-pharmacological strategy to control obesity and weight gain [16-18].

In addition, exercise can be used as a treatment to ameliorate the symptoms of many of obesity associated conditions, and thus the concept that exercise is medicine, Gomez-Merino et al., [19] is increasingly promoted in the hope that the general population can be persuaded to partake in more physical activity. It has been demonstrated that intermittent swimming exercise is more efficient than continuous swimming exercise in decreasing adiposity in rats fed a high-fat diet. Bradley et al., [15] (2008) showed that exercise could decrease visceral white adipose tissue inflammation in high-fat diet-induced obesity in mice. Moreover, Sakurai et al., [20] and Kawai et al., [21] had demonstrated that exercise can minimize inflammation even in non-obese rats.

Our hypothesis is that obesity-induced low grade inflammation could be reversed by daily swimming training exercise through decreasing expression and the circulatory levels of the inflammatory mediators and cytokines.

Material and Methods

Orlistat was purchased from Sigma Pharmaceutical Industries agency, KSA, as capsules, each capsule contains 120mg orlistat. ELISA kits for detecting rat serum leptin and ghrelin (Cat. No. ab 100773 and ab 120231, respectively) were purchased from Abcam Biochemicals, USA. ELISA kits for determination of serum levels of high sensitive C-reactive protein (hsCRP, Cat. No. ERC1021-1) was purchased from AS SAYPRO, USA. Serum Interleukin-6 (IL-6, Cat No. ELROI6-001) and Intracellular adhesive molecule (ICAM-1, Cat. No. ELR-ICAM-1-001) determination ELISA were purchased from RayBiotech, Inc, USA. Serum tumor necrosis factor alpha (TNF-α, Cat No. R63635) and vascular cell adhesive molecule (VCAM, Cat. No. R6148) determination ELISA kits were purchased from TSZ scientific, USA. Colorimetric Kits for determination of serum lipids including total triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL), and low density lipoprotein cholesterol (LDL) were purchased from Human Company, Germany.

Eight-week old male Wistar rats (n=40) weighing 280-300g were obtained from the rat’s breeding colony at the animal house of the college of medicine at King Khalid University, Abha, Saudi Arabia. Rats were housed in a 5 rat cages. Rats in all treatment groups were preconditioned for one week prior to implementing treatment protocol. During this period, rats received standard chow diet and water ad-libitum and were kept at a room temperature of 22±2°C, relative humidity of 55±10% and a light/dark cycle of 12 hours. All animals experimental procedures were conducted according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publications No. 85-23, revised 1996) as well as the protocol approved by King Khalid University Animal Care Committee. This study was designed and done in the period from June 2012 and June 2013.

Experimental design:

Rats were permitted to adapt for one week prior to protocol implementation. Four groups, 10 rats each, were formed; Group 1: Control (non obese rats): Rats which were fed standard diet (12% of calories as fat; Table 1) for 12 consecutive weeks; Group 2: HFD (obese rats): Rats which were fed high fat diet (HFD) (40% of calories as fat; Table 1) for 12 consecutive weeks Tuzcu et al., [22]. Group 3: HFD+Orlistat: Rats which were fed a high fat diet (40% of calories as fat) and received a concomitant therapeutic dose of orlistat (200 mg/kg) according to Nishioka et al., [23]. For 12 consecutive weeks; Group 4: HFD+Exercise: Rats which were fed a high fat diet (40% of calories as fat) and underwent a simultaneous daily swimming exercise (30min/day) for 12 consecutive weeks. Swimming exercise was done in a glass tank (dimensions: 100cm long, 40cm wide, 60cm deep) containing a 32°C tap water. Water depth in the tank was set at 30cm.

A- Body weight gain and biochemical analysis:

Body weight for all rats in every group was recorded before study initiation (Day 0) and at the end of week 12 of the protocol. Then, rats were anesthetized with diethyl ether and 3m1 blood samples were collected using 3m1 syringe directly from the heart using ventricular puncture method into plain 5m1 untreated glass tubes where they were allowed to clot for 15min at room temperature. Samples were centrifuged at 4000rpm for 10min to obtain the serum, which was used to determine the levels of TG, TC, HDL-cholesterol, LDL-cholesterol, ICAM-1, VCAM, 11-6, TNF-α, hsCRP, leptin and ghrelin, as per manufacturer’s kits’ instructions.
Adiposity index:

After blood collection, animals were sacrificed by decapitation, and adipose tissue was isolated and weighed from the epididymal, visceral and retroperitoneal pad. Adiposity index was determined by the sum of epididymal, visceral and retroperitoneal fat weights divided by body weight x100%, and expressed as adiposity percentage.

Statistical analysis:

Statistical analysis was performed by one way ANOVA. To identify the presence of any significant difference in the means, ANOVA testing was used followed by post hoc comparisons (Tukey's t-test). Data were expressed as means±standard deviation (SD) and statistical significance was set at the pD3.05 levels.

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<th>Table (1): Ingredients and nutrient composition of rat high fat diet.</th>
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Results

Fig. (1) showed that high fat diet (HFD) increased body weight of rats significantly (p<0.05) as compared with the control group. Orlistat treatment conjoined with HFD significantly (p<0.05) decreased the body weight as compared with the high fat diet group but still significantly (p<0.05) higher than the control group.

Additionally swimming exercise significantly (p<0.05) reduced the body weight as compared with the high fat diet and HFD+Orlistat but still significantly (p<0.05) more than the control group.

Fig. (2A) showed that the changes in the visceral, epididymal and retroperitoneal fat deposits in the different groups. HFD produced significant (p<0.05) increase in the all types of fat deposits as compared with the control group. Orlistat combination with HFD decreased the fat deposits significantly (p<0.05) as compared with the HFD fed group and insignificantly (p>0.05) with the control group. Also, exercise with HFD significantly reduced the fat deposits as compared with the HFD fed rats. Also, in response to exercise the epididymal and retroperitoneal fat deposits decreased significantly (p<0.05) as compared with HFD+Orlistat group with insignificant decrease in visceral fat.

Fig. (2B) showed that the adiposity index increased significantly in HFD fed rats, as compared with the control rats. HFD+Orlistat and HFD+Exercise rats showed a significant (p<0.05) reduction in the adiposity index as compared with the HFD fed rats.

Fig. (3) showed that HFD significantly (p<0.05) increased the lipid profile (serum cholesterol, triglycerides, HDL—cholesterol and LDL—cholesterol) as compared with the control group. HFD+Orlistat and HFD+Exercise significantly (p<0.05) reduced the lipid profile (serum cholesterol, triglycerides, HDL—cholesterol and LDL—cholesterol) as compared with the HFD fed rats.

Fig. (4A) showed the serum leptin significantly (p<0.05) increased in response to HFD as compared with the control rats. Serum leptin decreased significantly (p<0.05) in both HFD+Orlistat and HFD+Exercise rats as compared with the HFD fed rats but still higher than the control group.

Fig. (4B) showed that serum ghrelin decreased significantly in response to HFD as compared with the control group. Additionally HFD+Orlistat and HFD+Exercise significantly (p<0.05) increased the serum ghrelin level as compared with HFD fed rats.

Fig. (5A,B) showed that serum levels of ICAM-1 and VCAM significantly (p<0.05) increased in HFD fed rats as compared with the control group. Treatment of HFD fed rats with orlistat and exercise decreased significantly (p<0.05) both the ICAM-1 and VCAM as compared with the HFD fed rats.

Fig. (6A,B,C) showed that in response to feeding the rats with HFD, the serum levels of TNF-a, IL-6 and hs-CRP increased significantly (p<0.05) as compared with the control group. While treatment of the rats with exercise and orlistat significantly (p<0.05) reduced the plasma levels of the inflammatory markers as compared with the HFD fed rats.
Effect of Exercise & Orlistat Therapy in Rat Model

Fig. (1): Changes in body weight in the control and the experimental groups of rats. Values are expressed as Mean±SD for 10 rats in each group. Values were considered significantly different at p<0.05. (A)*: Significantly different when compared to same group initial weight. β: Significantly different when compared to HFD obese group. X: Significantly different when compared to HFD+Orlistat group. Obesity rat model. Photo (A): Rats received standard diet (Table 1); Photo (B): Rats received High Fat Diet (Table 1).

Fig. (2): Weights of different fat deposits and percents of adiposity (adiposity index) in the control and the experimental groups of rats. Values are expressed as Mean±SD for 10 rats in each group. Values were considered significantly different at p<0.05*: Significantly different when compared to control group I. β: Significantly different when compared to HFD+Orlistat group.

Fig. (3): Serum levels of total triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) in the control and the experimental groups of rats. Values are expressed as Mean±SD for 10 rats in each group. Values were considered significantly different at p<0.05*: Significantly different when compared to control group I. β: Significantly different when compared to HFD obese group. X: Significantly different when compared to HFD+Orlistat group.
Fig. (4): Serum levels of Leptin (A) and Ghrelin (B) in the control and the experimental groups of rats. Values are expressed as Mean±SD for 10 rats in each group. Values were considered significantly different at p<0.05*: Significantly different when compared to control group I. β: Significantly different when compared to HFD obese group. A.: Significantly different when compared to HFD+Orlistat group.

Fig. (5): Levels of intracellular adhesion molecule (ICAM-1, A) and vascular cell adhesion molecule (VCAM, B) in the serum of the control and experimental groups of rats. Values are expressed as Mean±SD for 10 rats in each group. Values were considered significantly different at p<0.05*: Significantly different when compared to control group I. β: Significantly different when compared to HFD obese group. A.: Significantly different when compared to HFD+Orlistat group.

Fig. (6): Levels of TNF-α (A), IL-6 (B) and hs-CRP (C) in the serum of the control and experimental groups of rats. Values are expressed as Mean±SD for 10 rats in each group. Values were considered significantly different at p<0.05*: Significantly different when compared to control group I. β: Significantly different when compared to HFD obese group. A.: Significantly different when compared to HFD+Orlistat group.

Discussion

In the present study the aim of work was to investigate the effects of feeding rats with HFD on the serum level of leptin, ghrelin and the inflammatory markers (ICAM-1, VCAM, TNF-α, IL-6 and hs-CRP). Also, treatment of the HFD fed rats with orlistat and exercise could modify the metabolic disturbances produced by high caloric intake in those rats.

Obesity, defined as a BMI >30kg/m2, is a global epidemic that currently affects over 185 million adults in industrialized nations, 115 million in the developing world and over 18 million children under the age of five. Patients who have a BMI >25 are considered overweight, while a BMI >30 is considered obese, and a BMI >40 is considered morbidly obese. In the US, more than 27% of the population is currently obese, with over half of the population being overweight. There are approximately 260,000 to 380,000 deaths a year from factors related to obesity in the US Allison et al., [24]. The BMI has served as a useful clinical tool in the evaluation of obesity and the appropriateness of patients for surgery. However, as a measure of total fatness, the BMI measurement fails because it underestimates the total amount of fat in females (females have more fat mass then men at similar BMI5) and overestimates fat mass in muscular men. Visser et al., [25]. A potentially useful addition to the appraisal of health risks of obesity would be biologic markers that assess the amount of excess inflammation.
In the present study, the high caloric intake in the form of high fat diet increased the body weight, fat content as well as the adiposity index. These results were in accordance with Matos et al. [26] and Rezq & El-Khamisy [27], who reported that the increase in liver weight could be a consequence of their higher fat content. Non-alcoholic fatty liver disease (NAFLD) is the build-up of extra fat in liver cells that is one of the characteristics of metabolic syndrome. It is normal for the liver to contain some fat. However, if more than 5%-10% percent of the liver’s weight is fat, then it is called a fatty liver (steatosis). It is complicated with steatohepatitis and liver cirrhosis. In addition, Matos et al. [26] and Rezq & El-Khamisy [27], observed intracellular lipid accumulation in cardiomyocytes in response to cholesterol diet. Treatment of the HFD fed rats with orlistat and exercise reduces the body weight, adipose tissue content and adiposity index. This can be explained by the reduction in the adipose tissue accumulation.

In the present study feeding rats with HFD increased the serum level of the inflammatory markers IL-6, TNF-a, hs-CRP, ICAM-1 and VCAM significantly. Until recently, it was accepted that the role of the adipocyte was the passive storage of energy in the form of white fat, or of brown fat for thermogenesis [28]. Our results were in accordance with previous studies that demonstrated that obesity is associated with low grade systemic inflammation and increased serum levels of acute phase proteins such as IL-6 and TNF-a [7].

The function of these cells was thought to be the release of fatty acids in times of starvation or the production of heat in times of cold, respectively. However, with the explosion of obesity-related research, it has become clear that fat cells actively monitor their environment, and vigorously respond to neural, paracrine, autocrine and endocrine inputs. These inputs include a wide array of steroids, cytokines, prostaglandins, cholesterol, and fatty acids [29]. The most notable inflammatory mediators released by the adipocyte are angiotensinogen (AGT), transforming growth factor beta (TGFβ), tumor necrosis factor alpha (TNFα), and interleukin six (IL-6).

TNF-a in obesity is produced by adipocytes throughout the body as seen in mRNA studies, but more abundantly by adipocytes in the waist-hip region [30]. This is manifested as a mild correlation of serum TNFa with BMI, but strong correlation with waist-hip ratio. (The waist is measured at the narrowest point of the relaxed stomach while the hips are measured on the widest place) [31]. Both the adipocyte mRNA expression and serum TNFa levels are elevated in obesity and concurrently decreased with weight loss.

Also, serum leptin increased significantly in obese rats in response to HFD. Leptin plasma concentration and mRNA expression in adipose tissue are directly related to obesity severity, as an increase of fat mass is associated with an increase of leptin which makes leptin an indicator of the total fat mass [32]. Also, our results showed that serum ghrelin decreased significantly in obese rats as compared with the control non-obese rats. These results were in agreement with Tschop et al. [33].

In the present work we investigated the effect of orlistat treatment with HFD on the serum lipids, leptin and ghrelin as well as the inflammatory cytokines. Orlistat treatment reduced the body weight, adipose tissues and serum lipids as compared with the HFD fed rats. Orlistat is a reversible lipase inhibitor that acts by inhibiting the absorption of dietary fats. Consequently with decreased body weight and total adipose tissue levels, the serum level of the inflammatory markers reduced significantly as compared with HFD fed rats. Also, serum leptin decreased in response to orlistat treatment with significant increase in the serum ghrelin level.

Regular physical exercise (five times/week) can delay or prevent the onset of type 2 diabetes in high-risk individuals with impaired glucose tolerance through increased glucose transport to the muscle fiber Kowler et al. [34]. At the same time physical activity is accompanied by reduction in the insulin plasma level that results in turning off different enzymes entangled in carbohydrate and lipid metabolism [35]. Lifestyle changes, including regular physical activity, have been reported to be more effective in preventing diabetes than drug therapy with metformin (Kowler et al., [34] 2002). This has primarily been attributed to the ability of exercise to improve a variety of metabolic abnormalities and risk factors that are associated with increased atherosclerosis. For example, aerobic exercise has been reported to lower blood pressure, improve dyslipidemia, facilitate weight loss, improve insulin sensitivity, and enhance glucose disposal, thereby reducing the incidence of diabetes [36-38].

In the present study swimming exercise reduced the body weight, adiposity index, serum cholesterol; triglycerides and LDL-cholesterol and increased HDL-cholesterol. These results were in accord with previous reports who concluded that regular exercise has long been associated with reduced cardiovascular risk. This has primarily been attributed to the ability of exercise to improve a variety
of metabolic abnormalities and risk factors that are associated with increased atherosclerosis. Exercise has been reported to decrease blood pressure, improve dyslipidemia, facilitate weight loss, improve insulin sensitivity, and enhance glucose disposal, thereby reducing the incidence of diabetes. It has been suggested that many of these improvements may be largely due to exercise-induced reductions in weight and insulin resistance [39].

Also, in response to swimming exercise the serum level of the inflammatory markers (IL-6, TNF-α, ICAM-1, VCAM and hsCRP) decreased significantly as compared with HFD fed rats. The reduction in adipokines production in response to exercise is studied by several researchers who concluded that exercise reduced the inflammatory state associated with obesity [40].

The circulating levels of leptin is concomitant with the degree of obesity and insulin resistance. Leptin plasma concentration and mRNA expression in adipose tissue are directly related to obesity severity, as an increase of fat mass is associated with an increase of leptin which makes leptin an indicator of the total fat mass. Leptin is considered as starvation hormone that was decreased with fasting and weight loss. In our study the level of leptin decreased significantly in response to exercise in HFD fed rats in agreement with the results obtained by Rajala et al., [41]. Decreased leptin could be attributed to the decreased body weight as well as the adipose tissue content.

Also, the serum level of ghrelin increased significantly with regular swimming exercise as compared with HFD fed rats. Ghrelin, a 28-amino acid peptide recently isolated from the human and rat stomach, is also present in human and rat pancreatic alpha-cells [42]. It is also recognized as a novel player in the gut-brain regulation of growth hormone and energy balance [43]. The molecule has been shown to be a growth hormone (GH) secretagogue that stimulates an increase in blood glucose [44,45]. Ghrelin has a potent effect on eating behavior, causing an increase in hunger and plays a key role in the central regulation of feeding [46]. It has been suggested that the stomach is a major source of circulating ghrelin in humans [47]. Our results were in agreement with results obtained by Ghanbari Niaki et al., [48].

Conclusion:

Exercise or orlistat treatment reduced the low grade inflammation induced in obesity by reducing the serum level of IL-6, TNF-α, hs-CRP, ICAM-1 and VCAM.

References


