Abstract. – Background: Obesity, a worldwide health problem, is a metabolic disease currently associated with a cluster and progressive pathologies presenting several features of metabolic syndrome.

Objectives: The present study was undertaken to investigate the effect of rimonabant, simvastatin and their combination on obesity associated metabolic disorder mediators in adult male rats.

Materials and Methods: Fifty adult male Wistar rats weighing (120 ± 10 g) were divided into five groups: Group 1 was kept on standard rodent chow and served as normal diet control. Group 2 was given high fat diet (HFD) for twenty weeks and served as HFD control. Groups 3, 4 and 5 administered HFD for ten weeks and then orally received rimonabant (2 mg/kg/day), simvastatin (10 mg/kg/day), combination of both drugs, respectively for another ten weeks with continuing feeding HFD.

Results: The current results showed that the treatment of HFD rats with either rimonabant or simvastatin significantly reduced body mass index, total cholesterol, triacylglycerides, low density lipoproteins, tumor necrosis factor alpha and monocyte chemoattractant protein-1, while increased adiponectin serum levels. Rimonabant showed to be more effective than simvastatin. Moreover, concomitant administration of rimonabant and simvastatin achieved the highest effect which nearly normalized most of the studied parameters as compared to singular therapy.

Conclusion: Rimonabant is the drug of primary choice as singular therapy for obesity. The adjunct therapy of rimonabant with simvastatin may be a novel and a promising therapeutic approach as it has a beneficial effect on the pathophysiological processes of obesity and its associated metabolic disorders.

Key Words: Rimonabant, Simvastatin, Obesity, Metabolic disorder.
crease in anti-inflammatory cytokine levels such as adiponectin. This obesity-associated inflammatory component seems to play an important part in the dramatic progression of obesity and metabolic syndrome. Moreover, obesity is associated with premature death and significant health care costs. Many obese patients have comorbidities that worsen their prognosis, particularly if hypercholesterolemia is present. In these patients, dietary restrictions are not sufficient to reduce hypercholesterolemia, lose body weight and ameliorate associated metabolic risks.

Rimonabant, the first cannabinoid receptor (CB1-receptor) blocker, is intended as an antiobesity and smoking-cessation dual-purpose drug. Rimonabant produces a dose-dependent reduction in food intake in various rodent models and these effects seem to be both centrally and peripherally mediated. Potential peripheral mechanisms include; (1) enhanced thermogenesis via increased oxygen consumption in skeletal muscle, (2) diminished hepatic and adipocyte lipogenesis, (3) augmented adiponectin concentrations, (4) promoted vagally mediated cholecystokinin-induced satiety, (5) inhibited preadipocyte proliferation, and (6) increased adipocyte maturation without lipid accumulation. Large-scale clinical trials have demonstrated that rimonabant therapy can reduce waist circumference, body weight and also improve several metabolic risk factors. Although, the precise mechanisms of action of rimonabant have to be further dissected, it is emerging, from both preclinical and clinical research, that not only is rimonabant an antiobesity drug, but also it functions as a drug for a broad range of diseases, from obesity-related comorbidities to drug dependence and cancer.

The principle goal of the present study was to investigate the potent influence of rimonabant, simvastatin and their combination on obesity-associated metabolic disorder mediators in adult male rats.

Materials and Methods

Experimental Animals

Fifty adult male Wistar rats of similar age and weight (120 ± 10 g) were enrolled in the present study. The animals were obtained from the Animal House Colony of the National Research Centre (Cairo, Egypt). They were housed in stainless steel wire meshed suspended rodent cages under environmentally controlled conditions. The ambient temperature was 25 ± 2°C and the light/dark cycle was 12/12 hours. The animals had free access to water and subjected to either a standard rodent chow diet or a high fat diet (HFD) as described below. All animals received human care in compliance with guidelines of the Ethical Committee of National Research Centre, Egypt.

Dietary Supplements

Standard laboratory chow (STD): fat content 10% protein 17% and carbohydrate 73%.

High-fat diet (HFD): fat content 49% (based on lard, olive oil and coconut oil), protein 18% and carbohydrate 18%.

Drugs

• Rimonabant (Acomplia® 20 mg tablets, Sanofi-Synthelabo, Montpelleir, France);
• Simvastatin (Zocor® 40 mg tablets, Merk, Canada).

Experimental Design

A week after acclimatization, the animals were randomly divided into five groups. Group (1) was kept on standard rodent chow for twenty weeks and served as normal control. Group (2) received high fat diet (HFD) for twenty weeks and served as HFD control. Group (3) was given HFD for ten weeks, then orally received rimonabant (2 mg/kg/day) for another ten weeks with continuing feeding HFD. Group (4) administered HFD for ten weeks, then orally received simvas-
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tatin (10 mg/kg/day) for another ten weeks with continuing feeding HFD. Group (5) was kept on HFD for ten weeks, then orally received a combination of rimonabant and simvastatin at the same doses mentioned above for another ten weeks, with continuing feeding HFD.

Weight gain and waist circumference were monitored once a week. Body mass index (BMI) was determined firstly at the end of the 10th week and secondly at the end of the 20th week. Blood samples were collected under anaesthesia from retro-orbital sinus in a dry centrifuge tube, centrifuged at 1400 × g for 15 minutes at 4°C. Aliquots of serum were frozen and stored at −20°C for further determinations of biochemical markers including: triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1) and adiponectin.

After blood collection, animals were sacrificed, visceral adipose tissue were removed, 10% Formalin-fixed and paraffin-embedded adipose tissue were processed. Four-µm-thick serial sections of adipose tissue were cut and stained with Oil-Red-O histopathological investigation using a light microscope.

Biochemical Analyses

Serum TG was determined by StanBio Laboratory kits (Boerne, TX, USA) according to Dryer29 based on the action of the enzyme lipoproteinlipase with liberation of glycerol and free fatty acids. Glycerol is converted into glycerol-3-phosphate and adenosine-5-diphosphate by glycerol kinase and ATP. Glycerol-3-phosphate is then converted by glycerol phosphate dehydrogenase into dihydroxyacetone phosphate and hydrogen peroxide. In the last reaction, hydrogen peroxide reacts with 4-aminophenazone and p-chlorophenol in the presence of peroxidase to give a red colored dye. The intensity of the color formed is proportional to the triglyceride concentration in the sample. Finally, colorimetric determination was performed in a spectrophotometer (Eppendorf, model Biophotometer, Hamburg, Germany) at a wavelength of 505 nm.

Serum TC was determined by StanBio Laboratory kits (Boerne, TX, USA) according to Stein30 based on the action of the enzyme cholesterol esterase which hydrolyzed the esters present in the sample, giving free cholesterol and fatty acids. A subsequent enzymatic oxidation using the cholesterol enzyme oxidase formed hydrogen peroxide and cholesterol. The peroxide was evaluated by the Trinder reaction by a chromogen, in the presence of peroxidase, forming a quinonimine with a red coloring. The intensity of this color was proportional to the cholesterol concentration in the sample. Finally, colorimetric determination was performed in a spectrophotometer (Eppendorf, model Biophotometer, Hamburg, Germany) at a wavelength of 500 nm.

Serum HDL was determined by StanBio Laboratory kits (Boerne, TX, USA) according to Finley et al31 based on the action of a product which induces the precipitation of the LDL- and VLDL-lipoproteins. Thus, the HDL-lipoproteins are only isolated in the supernatant, in which the binding of cholesterol is then measured. Finally, colorimetric determination was performed in a spectrophotometer (Eppendorf, model Biophotometer, Hamburg, Germany) at a wavelength of 500 nm.

Serum LDL was determined by StanBio Laboratory kits (Boerne, TX, USA) according to Steele et al32 with a two-step technique. First, chylomicrons, VLDL and HDL are eliminated as cholesterol. This cholesterol, specifically derived from these lipoproteins, but not from LDL, is oxidated to colesterine and hydrogen peroxide and then degraded to catalase. In a second reaction, LDL is specifically measured through the action of peroxidase with the formation of pinkish color quinine. The intensity of the color formed is proportional to the LDL concentration in the sample. Finally, colorimetric determination was performed in a spectrophotometer (Eppendorf, model Biophotometer, Hamburg, Germany) at a wavelength of 600 nm.

Serum TNF-α was determined according to Seriolo et al33 using solid phase Enzyme Linked Immuno Sorbent Assay (ELISA) by using TNF-α kits (Biosource International, Camarillo, CA, USA) and microtiter plate reader, Fisher Biotech, Ebersberg, Germany.

Serum MCP-1 was determined according to Ono et al34 by solid phase Enzyme Linked Immuno Sorbent Assay (ELISA) using MCP-1 kits (Biosource International, Camarillo, CA, USA) and microtiter plate reader, Fisher Biotech, Ebersberg, Germany.

Serum adiponectin was determined according to Watanabe et al35 by solid phase Enzyme Linked Immuno Sorbent Assay (ELISA) using adiponectin kits (Biosource International, Camarillo, CA, USA) and microtiter plate reader, Fisher Biotech, Ebersberg, Germany.
Statistical Analysis

The obtained data were statistically analysed. The differences between groups were assessed by one-way analysis of variance (ANOVA), using the SPSS software (Chicago, IL, USA). The values are expressed as mean ± SD. Inter-group comparisons were performed by Duncan’s multiple rank test using MSTAT-C computer program. Mean values followed by the same alphabetical letter are not significantly different. Differences were considered to be significant at $p < 0.05$.

Results

In the present study, feeding rats with high fat diet (HFD) for twenty weeks causes a marked increase in body weight by 35% as compared to normal control group kept on standard rodent chow for the same period. There was significant increase in waist circumference (39%) as well as BMI (34%) in rats supplemented with HFD as compared to normal control (Figure 1).

Regarding the effects of HFD on lipid profile, there were significant increases in serum TG (39%), TC (106%) and LDL (328%) accompanied by significant decrease in serum HDL (8%) in HFD group in comparison with the normal control group (Figure 2).

Serum levels of pro-inflammatory cytokine TNF-α and pro-atherosclerotic chemokine MCP-1 were significantly elevated in HFD group versus normal control group by 48% and 73% respectively. However, serum adiponectin was significantly reduced (47%) in HFD group compared to normal control group (Figure 3).

Supplementing HFD rats with 2 mg/kg rimonabant for ten weeks modulated body composition significantly by attenuating body weight and waist circumferences as well as body mass indices by 42%, 25% and 33% respectively as compared to HFD group (Figure 1). Rimonabant showed an ameliorative effect on dyslipidemia which was manifested in significant reductions in serum TG, TC and LDL levels, accompanied by a significant elevation in serum HDL level (23%) versus HFD group (Figure 2). In addition, rimonabant therapy significantly lowered serum MCP-1 level (41%), raised serum adiponectin level by 97% as compared to HFD group and almost normalized serum TNF-α level (Figure 3).

Administration of simvastatin (10 mg/kg) in HFD rats for ten weeks significantly reduced body weight (30%) and BMI (22%) versus HFD group. However, simvastatin failed to significantly alter waist circumferences (Figure 1). Analysing lipid profile after simvastatin treatment revealed that serum TG, TC, and LDL was significantly low-
Figure 2. Effect of Rimonabant®, Simvastatin® and their combination on lipid profile in rats. a, b, c, Columns followed by the same alphabetical letter are not significantly different at $p < 0.05$.

Figure 3. Effect of Rimonabant®, Simvastatin® and their combination on serum TNF-α (A), MCP-1 (B), and adiponectin (C) in rats. a, b, c, Columns followed by the same alphabetical letter for each parameter are not significantly different at $p < 0.05$. 
erred regards to HFD group, whereas it has null effect on HDL level (Figure 2). In addition, simvastatin therapy significantly down regulated both serum TNF-α and MCP-1 levels by 27% and 24% respectively as compared to HFD group. Meanwhile, it significantly raised serum adiponectin level by 46% versus HFD group (Figure 3).

Co-administration of rimonabant and simvastatin significantly reduced body weight (49%) and waist circumferences (23%) as well as BMI (34%) as compared to HFD group (Figure 1). Dyslipidemia was significantly improved after the combination therapy and this was evidenced by significant depression in serum TG (24%), TC (50%) and LDL (80%) levels, whereas serum HDL was significantly higher by 25% than HFD group (Figure 2). Rimonabant and simvastatin combination significantly reduced serum TNF-α (44%) and MCP-1 (43%) levels, meanwhile, this combined therapy upregulated serum adiponectin level by 91% versus HFD group (Figure 3).

Histopathological examination of adipocytes of HFD rats revealed great deposition of lipid droplets in the cytoplasm as compared to the normal control. Treatment of HFD rats with simvastatin and/or rimonabant showed less deposition of lipid droplets in the cytoplasm of the adipocytes as compared with the adipocytes of HFD rats. The data revealed that, lipid deposition in the adipocytes of either rimonabant alone or in its combination with simvastatin was lesser than that in the adipocytes of simvastatin treated group (Figure 4).

**Discussion**

Obesity is an inflammatory chronic and progressive disease, characterized by an increased body weight and development of adipose tissue

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**Figure 4.** This figure shows histopathological examination of adipose cells of HFD rats treated with simvastatin® or rimonabant® and their combination (oil red-O × 200). A, Adipocytes of normal rat. B, Adipocytes of HFD rats. C, Adipocytes of simvastatin. D, Adipocytes of rimonabant. E, Adipocytes of combined therapy.
with excessive fat storage. In the present study, feeding rats with HFD for twenty weeks significantly increased body weight, waist circumference as well as BMI as compared to the normal diet control rats. Adipose tissue is a dynamic tissue and an important endocrine organ, secreting biologically active molecules called adipocytokines that are involved in the regulation of energy metabolism and body weight homeostasis. It has been reported that these adipocytokines play a crucial role in several pathophysiological processes, including obesity and associated diseases.

High fat diet induced obesity is also recognized as a major risk factor for lipid disorders including hypercholesterolemia, reduced HDLc/LDLc ratio, increased inflammation and a procoagulant state. These previous findings are in consistent with the results obtained in the current study, where HFD significantly elevated serum TG, TC and LDL levels, whereas decreased serum HDL level. These results could be discussed on the basis that the intestinal absorption capacity can be adapted to the fat content of the diet. This fat-mediated adaptation takes place through the enhancement of the intestinal cell proliferation, which might lead to an increased absorptive area. Consistent with this assumption, rat studies showed that fatty acid-enriched diets increase the height of villi and induce the rate of enterocyte migration along the crypt-to-villus axis. Moreover, fat-induces coordination of genes known to play a significant role in intestinal fatty acid uptake or intracellular processing and lipoprotein secretion. HFD can promote a down regulation of hepatic LDL receptor, leading to a decrease in hepatic clearance of LDL. Hypertriglyceridemia resulting from HFD may be due to increased hepatic triglycerides production and VLDL secretion, as a consequence of increased adipocyte hormone sensitive lipase activity and decreased muscle lipoprotein lipase activity.

The present investigation demonstrated that HFD significantly elevated pro-inflammatory cytokine (TNF-α) and pro-atherogenic chemokine (MCP-1). These results agree with Morin et al and Schafer et al. Adipose tissue is a source of TNF-α (AT-TNF) as the elevated levels of AT-TNF protein and mRNA in adipose tissue were originally observed in rodents models of obesity. AT-TNF mRNA was decreased following weight reduction. So AT-TNF activity as well as protein and mRNA is increased by high fat feeding.

Obesity is a complex metabolic disorder which contributes to lipid deposition in the arterial wall and initiate leukocyte and monocyte recruitment. The monocyte chemoattractant protein recruit monocytes to the vascular endothelium, where they enter the subendothelial space, accumulate lipids and differentiate into macrophages. MCP-1 is present in these macrophage-rich atherosclerotic plaques and oxidized LDL which induces the production of MCP-1 in endothelial and smooth muscle cells. Obesity is a whole inflammatory state with an increased in circulatory level of pro-inflammatory cytokines such as TNF-α, C-reactive protein, interleukin 1β and a decrease in anti-inflammatory cytokine level as adiponectin. These findings agree with the present results which showed a significant depletion in serum adiponectin level in HFD rats. Several studies in animal models and human, have reported that adiponectin levels are inversely correlated with the increase in pro-inflammatory cytokines particularly TNF-α. Adiponectin and TNF-α antagonise each other in adipose tissue which may progress and induce serious pathologies as dyslipidemia, hepatic diseases and cardiovascular disorders.

There is no pharmacological agent that has both antiobesity effects and reverses obesity related features of metabolic disorders and their complications, in particular, the inflammatory component associated with metabolic diseases. The selective cannabinoid receptor type 1 (CB1 receptor) antagonist rimonabant has been reported to have potent antiobesity effect. The present study revealed that rimonabant treated rats showed significant reductions in body weight, waist circumference and BMI. These results are in accordance with the findings of Leite et al. The reduction in body weight and BMI may be partly a result of its peripheral metabolic action on adipose tissue, where rimonabant inhibits preadipocyte cell proliferation and induces a uncoupling of the association between the inhibition of adipocyte cell proliferation and lipid accumulation. This powerful property may account for the potent activity of rimonabant in obesity treatment.

The current results showed that rimonabant treatment produced significant reductions in TG, TC and LDL, whereas elevated protective HDL in HFD rats. These rimonabant induced changes in lipid profile are in the same line with Padwal and Majumdar, and Florentin et al and may be related to our observation that rimonabant treatment increases serum adiponectin levels. Where adiponectin level is significantly and positively correlated with HDL level and nega-
tively correlated with TC and LDL levels. Also, rimonabant can decrease lipoprotein lipase activity in primary adipocyte cell culture and, therefore, reduces body fat stores.

Our results demonstrated a significant decrease in serum TNF-α and MCP-1, whilst a significant elevation in serum adiponectin was detected following treating rats with rimonabant. These results agree with Lee and Pratley et al. and Schafer et al. The elevation in adiponectin is attributed to the stimulation of adiponectin mRNA expression in adipose tissue by a direct effect of rimonabant on adipocytes and also inhibition of mitogen-activated protein (MAP) kinase activity which attenuates the stimulation of adiponectin expression. These effects of rimonabant may be mediated via antagonism of local endocannabinoid tone or through the inverse agonist activity of this compound and involve functional CB1 receptors expressed in adipocytes. Adiponectin is inversely correlated with pro-inflammatory cytokines in particular TNF-α. The increase in serum levels of adiponectin along with the corresponding reduction in TNF-α underlie a large part of rimonabant anti-inflammatory effects. Rimonabant positively modulated obesity induced inflammatory pathway by reducing the substrate LDL, lowering levels of chemoattractant MCP-1 and decreasing circulating monocytes. This mechanism may be due to alternative signaling through CB2 receptors in the presence of functional CB1 blockade, where activation of the other endocannabinoid receptor CB2 reduces monocyte chemoattractant properties and monocyte-endothelial cell adhesion. CB2 receptors are found in immune cells and macrophages. This localization is directly coupled to the action of these receptors in the suppression of pro-inflammatory cytokines expression.

The current results clearly demonstrated the beneficial multiproteic effects of rimonabant therapy, particularly in reducing BMI, modulating lipid profile disorders, downregulating pro-inflammatory cytokines (TNF-α), attenuating the release of pro-atherogenic chemokines (MCP-1) and increasing protective anti-inflammatory cytokines and adiponectin.

Simvastatin, a 3-hydroxy-3-methyl glutaryl co-enzyme A (HMG-coA) reductase inhibitor is used in the treatment of hypercholesterolemia. In the present study, administration of simvastatin (10 mg/kg) in HFD treated rats significantly reduced body weight and BMI of HFD treated rats. These results were supported by the findings of Derosa et al. Also, our results revealed that simvastatin therapy significantly improved lipid disorders by lowering serum TG, TC and LDL levels but failed to significantly alter serum HDL level. These results are in agreement with Cavallini et al. Simvastatin inhibits HMG-coA reductase, the rate limiting enzyme of the mevalonate pathway, thereby, decreasing hepatic intra-cellular cholesterol synthesis resulting in compensatory increase in the expression of hepatic LDL receptors, which clear LDL from the circulation. Moreover, the drug reduces VLDL and triglycerides.

Our data showed that treating HFD rats with simvastatin significantly downregulated serum TNF-α and MCP-1, whilst elevated adiponectin level modestly but significantly as compared to HFD group. These results are in accordance with Park et al. and Liu et al. Simvastatin has direct inhibitory effect on TNF-α induced monocyte-endothelial cell adhesion which is mediated via geranylgeranyl isoprenoid-dependent generation ROS. Simvastatin may influence cell functions through diverse receptors and multiple pathways. It inhibits the activation of nuclear factor kappa B which is a major nuclear factor that regulates the expression of diverse cytokines including MCP-1, IL-8 and IL-6 mRNA as well as TNF-α induced expression of MCP-1, IL-8 and IL-6 mRNA. The mechanism by which simvastatin increases adiponectin concentration is unknown. However, it was observed that adiponectin concentration is associated positively with HDL-cholesterol and negatively with triglycerides and cholesterol/HDL-cholesterol at baseline. Hyperlipidemia increases the oxidative stress-reactive oxygen species (ROS) in the vessel wall by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase, leading to dysequilibrium of NO/O2- and the degradation of nitric oxide (NO) bioavailability. However, simvastatin therapy significantly increased NO levels, and reduced MDA levels. Adiponectin showed positive correlation with NO. This positive correlation between adiponectin and NO suggests that simvastatin has a therapeutic function through the restoration of normal circulating adiponectin.

Simvastatin confirmed its effectiveness in the management of blood lipid, modulation of pro-inflammatory cytokines and chemokines and also in increasing adiponectin. However, its effect was less pronounced than rimonabant therapy.

Co-administration of rimonabant and simvastatin produced significant improvement in
all measured parameters as compared to HFD group. The combination treatment showed significant reductions in body weight, waist and thoracic circumferences as well as BMI as compared to HFD treated group. Also the combination significantly lowered serum TG, TC and LDL levels, whilst significantly increased serum HDL level. Moreover, the combination therapy significantly lowered body weight, waist and thoracic circumferences as well as BMI as compared to HFD treated group. The combination treatment showed significant reductions in body weight, waist and thoracic circumferences as well as BMI as compared to HFD treated group. Also the combination showed the highest effect which nearly normalizes most of the studied parameters as compared to individual therapy.

The above mentioned biochemical results were clearly documented by the histopathological investigation of visceral adipose tissue sections of the different studied groups.

The present study concluded that the selective CB1 antagonist rimonabant and the HMG-coA reductase inhibitor simvastatin have antiobesity, anti hyperlipidemic and anti-inflammatory effects, where each of these drugs significantly reduced body weight, BMI, TC, TG, LDL, TNF-α and MCP-1 whereas significantly increased adiponectin.


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