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Effects of forskolin and rolipram on serum leptin, resistin and adiponectin levels in diet induced obesity in Wistar rats

Forskolin ve rolipramın Wistar sıçanlarında diyetle indüklenen obezitede serum leptin, rezistin ve adiponektin seviyeleri üzerine olan etkileri

Abstract: Objective: Forskolin, an activator of adenylate cyclase and rolipram a selective inhibitor of phosphodiesterase 4, stimulate lipolysis and inhibit body weight increase by increasing cyclic adenosine monophosphate (cAMP) levels. This study was aimed to investigate the effects of them on leptin, resistin and adiponectin levels in diet induced obesity in rats.

Methods: Totally 50 rats were randomly divided into five groups. The Group I was fed with standard pellet diet and the other groups were fed with high-fat diet for 10 weeks. During the last two weeks of the study, group II continued to fed with high-fat diet whereas group III, group IV and group V were administered forskolin, rolipram and forskolin plus rolipram respectively by orogastric tube in addition to their high-fat diet. Then, rats were sacrificed and serum leptin, resistin and adiponectin levels were measured.

Results: Although adiponectin levels of group II (p<0.001 for adiponectin, p<0.01 for leptin), group III (p<0.01), group V (p<0.05) were significantly decreased, leptin levels were significantly increased compared to that of the group I. Also, leptin levels of group IV were significantly reduced compared to those of group II (p<0.05). There were no significant differences between resistin levels of the groups.

Conclusion: Our results showed that rolipram prevented any alteration in the levels of leptin and adiponectin in addition to its effect on cAMP levels. However, forskolin and rolipram showed no effect on resistin levels of the groups. The underlying mechanism of these findings is not known and needs to be more investigated.

Keywords: Forskolin, rolipram, leptin, resistin, adiponectin, obesity, wistar rat

Özet: Amaç: Bir adenilat siklaz aktivatörü olan forskolin ve bir selektif fosfodiesteraz 4 inhibitori olan rolipram siklik adenozin monofosfat (cAMP) seviyesini artırarak lipolizi stimule ve viçut ağırlık artışını inhibe ederler. Bu çalışmada forskolin ve rolipramın sıçanlarda diyetle indüklenen obezitede leptin, rezistin ve adiponektin üzerine olan etkilerinin araştırılması amaçlanmıştır.

Metod: 50 sıçan randomize olarak 5 gruba ayrıldı. Grup I standart pellet diyet ile diğer gruplar ise yüksek yağlı diyetle 10 hafta beslendi. Çalışmanın son iki haftasında grup II yüksek yağlı diyet ile beslenmeye devam ederken, grup III, grup IV ve grup V ye yüksek yağlı diyete ilave olarak sırası ile forskolin, rolipram ve forskolin ile rolipram orogastrik tüp ile verildi. Sonra sıçanlar sakrifiye edildi ve serum leptin, rezistin ve adiponektin seviyeleri ölçüldü.

Bulgular: Grup I ile kıyaslandığında Grup II (adiponektin için p<0.001, leptin için p<0.01), grup III (p<0.01) ve...
Introduction

Obesity is a major health problem with a worldwide prevalence of about 7%. There are several factors that contribute to obesity such as genetic factors, physical activities, dietary habits, and neurohormonal imbalances [1,2]. Obese patients have an increased mortality risk due to cardiovascular complications [3].

Obesity is associated with elevated plasma free fatty acid (FFA) concentrations caused by impaired insulin suppression of adipocyte lipolysis [4,5]. It is currently argued that adipose tissue is not only a source of energy but also an active hormone system involved in metabolic control. Therefore, adipose tissue is now considered an important endocrine organ, which plays important roles in the regulation of insulin action, inflammation, haemostasis and other physiological processes [6]. Indeed, recent researches have demonstrated that adipose tissue produces and secretes various bioactive substances, conceptualized as adipocytokines, and their dysregulation in abdominal or visceral obesity may participate in the development of the metabolic syndrome [6-8].

Adipocytokines may affect the functioning and the structural integrity of other tissues. Adipocytokines include leptin, adiponectin, resistin, adiponutrin, acylation stimulating protein (ASP), tumour necrosis factor-α (TNF-α), plasminogen activator inhibitor (PAI-1), angiotensin II, interleukin-6 (IL-6) and soluble preadipocyte factor. Some of these cytokines, namely leptin and adiponectin and TNF-α appear to be involved in the development of obesity [6].

Leptin, is a satiety hormone predominantly produced from adipose tissue. Plasma leptin concentration is proportional to the amount of adipose tissue and is markedly increased in obese individuals. It has been proposed to play important roles in regulating body energy balance. The impairment of this energy balance leads to obesity [9]. Recent studies suggest that leptin contributes to the pathogenesis of arterial hypertension associated with obesity, one of the most common types of hypertension in developed countries [10].

Resistin also known as adipose tissue-specific secretory factor (ADSF) is a cysteine-rich protein [8]. Resistin is a peptide hormone produced by mature adipocytes and regulates whole-body insulin sensitivity [11]. Indeed, it has been reported that resistin impairs glucose tolerance and insulin action in mice [12] and a significant correlation between resistin levels and insulin resistance was also observed in humans [11]. Some studies on the regulation of resistin indicated that mRNA expression and protein production were reduced by fasting and rapidly increased on refeeding. However, in the literature, there are some conflicting results concerning diet-induced obesity in animals [12-15]. For example, Way et al. [14] and Le Lay et al. [15] reported a decrease in resistin mRNA level whereas Steppan et al. [13] found an increase in resistin serum concentration in such models. In humans, it has been reported that resistin is undetectable or weakly expressed in subcutaneous adipose tissue biopsies from lean subjects, and consistently present only in morbidly obese subjects [8].

Adiponectin, a collagens-like plasma protein is produced by adipose tissue [8]. Plasma levels of adiponectin were found to be decreased in obese patients [16] and type 2 diabetic patients [17] and increased after bodyweight reduction. Thus, its production was suggested to be under feedback inhibition in obesity [18]. Recent observations suggest an important role of adiponectin in the development of insulin resistance [8]. Clinical and experimental data suggest that adiponectin may play a significant role in the development of the metabolic syndrome [7].

Also, it has been reported that adiponectin suppresses almost all processes involved in atherosclerotic vascular change, including the expression of adhesion molecules in vascular endothelial cells, proliferation of vascular smooth muscle cells, and formation of foam cells in vitro, and it exhibits anti-atherosclerotic activity in vivo [7].

Forskolin, isolated from the roots of an Indian plant, coleus forskohlii (CF), is a diterpene which acts directly on
adenylate cyclase (AC) enzyme binding to a hydrophobic pocket close to the catalytic site of the enzyme [19]. Indeed, forskolin is a very effective activator of ACs 1 to 8 but not of AC9 [20,21]. CF has been reported to prove of value in the clinic for prevention of cancer metastasis [22]. Forskolin stimulates lipolysis via increasing cyclic adenosine monophosphate (cAMP) accumulation [23]. Therefore, it was reported that forskolin may be used as a therapeutic agent for weight management and treatment [24].

Rolipram is a selective phosphodiesterase (PDE) 4 inhibitor and reversibly binds with high affinity to the active PDE4 holoenzyme conformation. PDE4 selectively hydrolyzes cAMP to AMP [25-27]. Inhibition of PDE4 by rolipram prevents diet-induced obesity and lead to an increase in mitochondrial function, physical stamina, and glucose tolerance in mice. Also, the administration of PDE4 inhibitors may protect against and ameliorate the symptoms of metabolic diseases associated with aging [26].

The effects of forskolin and rolipram on obesity via cAMP levels have been investigated in various studies [24,26]. Indeed, in our previous study, we have found that both forskolin and rolipram stimulated lipolysis and inhibited body weight increase by increasing cAMP levels. Also, combination therapy using the two agents may be more effective in preventing diet induced obesity than either agent alone [28]. However, we haven’t encountered any study measuring the effects of these two substances on leptin, resistin and adiponectin. Therefore, in this study we have aimed to investigate the effects of forskolin and rolipram on obesity in rats.

Materials and Methods

The study was performed on 50 Albino Wistar female rats (250-300g) supplied by the Medical Experimental Research Centre (University of Selcuk, Konya, Turkey). The study protocol was approved by the Ethical Committee of the Selcuk University Experimental Medicine Research and Application Center.

All animals were housed in cages under control conditions of temperature (21±2°C), relative humidity (50±5%) and 12 hours light cycle (8 a.m. to 8 p.m.). All rats were given standard rat chow and tap water ad libitum for one week at the beginning of the experiment.

Then, rats were randomly divided into five groups as follows:

Group I (control group), group II (high fat diet group), group III (high fat diet plus forskolin group), group IV (high fat diet plus rolipram group) and group V (high fat diet plus rolipram plus forskolin group).

The control rats were fed a standard pellet diet (Nukleon; Ivedik Organize San. Bol. Ozankara, Ankara, Turkey) and the other groups were fed a high fat diet prepared in house by adding lard to the standard diet. Standard pellet composition was 24% protein, 3.62% fat, 7% cellulose, 10% ash and 12% water. Sixty percent of total energy was from fat in the high fat diet. Diets with 60% of energy from fat commonly are used to induce obesity in animals [29]. We added lard to the standard feed to produce a saturated high fat diet.

During the last two weeks of our study, forskolin (10% forskolin extract 5 mg/kg/day) was administered by orogastric tube to the group III and rolipram (0.1mg/kg/day) was administered to the group IV. Rats in the group V were administered forskolin plus rolipram (10% forskolin extract 5 mg/kg/day and 0.1 mg/kg/day rolipram) by orogastric tube. Weight gain of the animals were recorded regularly. Forskolin (Thorne Research - Coleus forskohlii, code number: SF756) and rolipram (Sigma, cat. number: R6520) were purchased from commercial sources.

Twenty four hours after the last doses of the substances, rats were sacrificed under ketamine/xylazine anesthesia and blood samples were collected by intracardiac puncture. 12 hours before euthanasia the groups were fasted by food withdrawal. Blood samples obtained from the rats were centrifuged at 1500 g at 4°C for 10 minutes, and sera were stored at -80°C until the day of biochemical analysis.

Serum leptin, resistin and adiponectin levels were measured by commercially available kits based on enzyme-linked immunosorbent assay (ELISA) methods under fasting conditions. Leptin and resistin levels were measured using commercially available ELISA kits from BioVendor, Czech Republic (Cat. no: RD291001200R for leptin and Cat. no: RD391016200R for resistin). Adiponectin levels were measured using commercially available ELISA kit from Shibayagi (Code no: AKMAN-011, Japan). The intra-assay and inter-assay coefficients of variance (CV) for leptin were 1.9% and 4.4%, for resistin were 5.0% and 7.1% and for adiponectin were 1.5% and 1.8%, respectively.

Statistical analysis

SPSS 16.0 statistical package program was used for all data analyses. Distribution characteristics of the variables were tested with Kolmogorov Smirnov test and Test of Homogeneity of Variances. Since the data were normally
distributed, we performed the parametric ANOVA-Tukey test to determine the differences between resistin, adiponectin and weight changes of the groups. Non-normally distributed leptin levels were examined by Kruskal-Wallis Test and then by Mann-Whitney U test. The results were given as mean±standard deviation (SD). The differences between the parameters were considered significant at a probability level of \( p < 0.05 \).

**Results**

Body weights and body weight changes in all groups at the beginning of the study and 10 weeks later were shown in Table 1 and body weights findings were compared in Figure 1. There were no significant differences between body weights of the groups at the beginning of the study. Body weight increase was 6.89 percent for group I, 19.78 percent for group II, 13.95 percent for group III, 14.94 percent for group IV and 7.55 percent for group V 10 weeks later. Body weight at 10 weeks later were significantly higher in group II compared to group I (\( p < 0.01 \)) and group V (\( p < 0.05 \)). Body weight changes were significantly higher in all groups except group V compared to group I (\( p < 0.001 \) for group II and group IV and \( p < 0.01 \) for group III). Body weight changes in group III (\( p < 0.01 \)), group IV (\( p < 0.05 \)) and group V (\( p < 0.001 \)) were significantly lower than those in group II. Also, body weight changes were significantly lower in group V compared to group III and group IV (\( p < 0.01 \)).

**Table 1:** Body weights and body weight changes of the groups at the beginning of the study and 10 weeks later.

| Parameters          | Group I          | Group II         | Group III        | Group IV         | Group V          |
|---------------------|------------------|------------------|------------------|------------------|------------------|
| First weight (g)    | 270.1±14.37      | 272.0±18.28      | 274.9±33.60      | 268.6±35.28      | 272.8±29.87      |
| Last weight (g)     | 290.1±14.18      | 339.1±22.55      | 319.5±36.63      | 315.8±35.8       | 295.1±30.97      |
| Weight increase (g) | 20.0±1.26        | 67.1±15.47       | 44.6±9.11        | 47.2±16.95       | 22.3±18.09       |
| Percent of weight increase (%) | 6.89            | 19.78            | 13.95            | 14.94            | 7.55             |

Group I refer to control group, group II refer to high fat diet group, group III refer to high fat diet plus forskolin group, group IV refer to high fat diet plus rolipram group and group V refer to high fat diet plus rolipram plus forskolin group.

**Table 2:** Leptin, resistin and adiponectin levels of the groups at the end of the study.

| Parameters          | Group I          | Group II         | Group III        | Group IV         | Group V          |
|---------------------|------------------|------------------|------------------|------------------|------------------|
| Leptin (pg/ml)      | 557.1±223        | 1722.85±161      | 1045.71±364      | 780.00±377       | 1171.42±697      |
| Resistin (ng/ml)    | 4.10±0.43        | 4.57±0.85        | 4.72±0.51        | 4.68±0.39        | 4.52±0.53        |
| Adiponectin (ug/ml) | 8.71±1.83        | 6.31±0.52        | 6.68±0.73        | 7.26±1.48        | 7.16±0.63        |

Group I refer to control group, group II refer to high fat diet group, group III refer to high fat diet plus forskolin group, group IV refer to high fat diet plus rolipram group and group V refer to high fat diet plus rolipram plus forskolin group.

Figure 1: Comparison of body weight changes of the groups. \( a \ p < 0.01, \ b \ p < 0.05 \) compared to group I and \( c \ p < 0.001, \ d \ p < 0.01, \ e \ p < 0.05 \) compared to group II and \( f \ p < 0.01 \) compared to group V.

Figure 2: Comparison of leptin, resistin and adiponectin levels of the groups (Leptin levels were multiplied by 0.01). \( a \ p < 0.001, \ b \ p < 0.01, \ c \ p < 0.05 \) compared to group I and \( d \ p < 0.05 \) compared to group II.
in Table 2 and compared in Figure 2.

Leptin levels of group II ($p<0.01$), group III ($p<0.01$) and group V ($p<0.05$) were significantly increased compared to that of the control group (group I). There was no significant difference between leptin levels of group IV and the control group. Also, leptin levels of group IV were significantly reduced compared to leptin levels of group II ($p<0.05$).

Adiponectin levels of group II ($p<0.001$), group III ($p<0.01$) and group V ($p<0.05$) were significantly decreased compared to that of the control group (group I). There was no significant difference between adiponectin levels of high fat group and high fat plus forskolin or rolipram administered groups.

There were no significant difference between resistin levels of the groups. Thus, neither high fat diet nor forskolin or rolipram administration caused any change in resistin levels.

**Discussion**

Our findings demonstrate that leptin levels were increased and adiponectin levels were decreased in high fat diet group (group II) compared to the control group (group I). It is known that leptin is increased [11,30,31] and adiponectin is decreased [6,11,16,30,31] in obese subjects. Therefore, our finding is in accordance with those of the above literature. However, in our study, forskolin displayed no effect on these alterations.

We have found no alterations in leptin, adiponectin and resistin levels of group IV (rolipram group) compared to that of the control group. This finding shows that rolipram prevented any alterations in leptin and in adiponectin levels in high fat group. Although, the underlying mechanism of this finding is not known it can be argued that rolipram might display that effect as a result of its anti-inflammatory effect [27,32].

On the other hand, we could not interphase high leptin levels found in forskolin plus rolipram group compared to that of rolipram group alone. Zhang et al. [32] have found no difference between leptin levels of PDE type 4B depleted rats fed with normal and high fat diet for a period of 10 weeks. This finding support our finding. Because, in our study, in spite of high fat diet, we have found no difference between leptin levels of rats fed with rolipram which is an inhibitor of PDE type 4.

Resistin is a hormone released from adipose tissue and associated with obesity [13] and diabetes [12]. However, physiological role of resistin in circulation is not known [33]. In our study, we have found no significant difference between resistin levels of the groups (forskolin, rolipram, or forskolin plus rolipram groups). Therefore, this finding shows that forskolin and rolipram have no effect on resistin levels.

We have found no study investigating the effects of rolipram or forskolin on adipocytokines. Therefore, this the first study investigating the effects of these two substances on these adipocytokines in diet induced obesity.

However, in the literature there are some studies investigating the change in adipocytokines levels in obesity. For example, Wolfe et al. [33] have found that in 4 weeks calory restricted healthy subjects leptin and adiponectin levels were significantly reduced but resistin levels were not changed.

However, Guéguenon et al. [34] have found that, in obese adolescents, a long-term combination of aerobic exercise and a balanced diet, inducing change in body composition and improved insulin sensitivity, markedly increased high molecular weight (HMW) adiponectin compared with total adiponectin, without any change in resistin concentrations.

Lee et al. [35] have reported no correlation between resistin levels and adipose tissue, body mass index (BMI), waist-to-hip ratio and fat mass. Also, they have found no correlation between resistin levels and insulin resistance, lipid profile and serum leptin levels. Thus, they concluded that resistin has no significant effect on obesity, insulin resistance and energy homeostasis in humans.

Silha et al. [11] have measured adiponectin and leptin levels in obese subjects and found that adiponectin levels were significantly lower, leptin levels were higher than those of the normal weight subjects whereas there was no difference between resistin levels of the groups. Yang et al. [36] have found that plasma resistin levels were notably elevated after feeding mice with a high fat-high sucrose diet for 4 weeks.

Normally, body weight reduction increases circulating adiponectin levels [37,38]. Indeed, a 21% reduction in BMI was found to cause a 46% increase in adiponectin levels in obese subjects who received gastric partition surgery [18]. Gil-Campos et al. [6] have found that plasma adiponectin levels were reduced with obesity both in adult and children and showed a negative correlation with insulin levels and a positive correlation with triglyceride levels. Thus, they suggested that adiponectin might be promising agent in the treatment of obesity and related metabolic disorders.

Bullen et al. [39] have reported that serum adiponectin levels were reduced in high fat fed rats resistant or vulnerable to diet induced obesity.
Milan et al. [8] have found that adiponectin and resistin levels gen expressions were significantly lower in fat tissue of obese rats than those the control group. These findings suggest that any treatment increasing adiponectin levels could be useful in the prevention of obesity.

One of the limitation of our study was the unknown effect of ketamine/xylazine anesthesia on leptin, adiponectin and resistin levels. Mastronardi et al. [40] have found that leptin gradually declined from pre-operative concentrations, reaching a minimum at 3 hr after high dose of ketamine/acepromazine/xylazine anesthesia. However, we did not find any literature reporting the effect of ketamine/xylazine anesthesia on resistin and adiponectin levels.

In summary, our results suggest that in diet induced obesity in rats leptin levels were increased and adiponectin levels were decreased in spite of administration of forskolin. However, rolipram has prevented alterations in the levels of these adipocytokins in the same conditions. Also there was no change in resistin levels of high fat group and forskolin or rolipram groups.

Our results showed that rolipram prevented any alteration in the levels of leptin and adiponectin however forskolin showed no effect on the levels of them. Also, forskolin and rolipram did not affect on resistin levels of the groups. Our findings suggest that the effects of rolipram and forskolin on diet induced obesity is important also in terms of the levels of adipocytokins in addition to their effect of cAMP and needs to be more investigated.

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Conflict of Interest: The authors have no conflict of interest.

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