

# EFFECTS OF YOHIMBINE ON PLASMA LEVELS OF LEPTIN IN NORMAL AND STREPTOZOTOCIN INDUCED DIABETIC RATS

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**Abstract-** Leptin affects paraventricular nucleus of hypothalamus and reduces appetite while activation of  $\alpha_2$ -adrenoceptors at this site has opposite action. The reducing effect of  $\alpha_2$ -adrenoceptors inhibition on body weight and appetite and also enhancing effects on lipolysis, sympathetic activation and plasma insulin levels in animals have been reported. We studied the effect of yohimbine treatment (2 mg/kg/day orally) as  $\alpha_2$ -adrenoceptor antagonist on plasma levels of leptin, insulin and glucose and also body weight in rats. Five normal yohimbine treated, five diabetic insulin treated (10 u/kg/day) and five diabetic insulin (10 u/kg/day) and yohimbine (2 mg/kg/day) treated male Sprague Dawley rats were used and treatment continued for six days. One blood sample before treatment and three blood samples after treatment (with one day interval) were collected from all rats. Our results showed statistically significant increase in leptin ( $P < 0.037$ ) and insulin ( $P < 0.042$ ) and decrease in body weight ( $P < 0.004$ ) in normal yohimbine treated rats. In diabetic rats insulin levels before and after treatment were similar in two groups but leptin, glucose and body weight were significantly reduced in yohimbine and insulin treated compared with just insulin treated rats. The present results indicate that yohimbine treatment can reduce body weight by increasing stimulation of lipolysis and increasing plasma levels of leptin. This plasma leptin enhancement in normal rats may be contributed to the weight reducing effect of  $\alpha_2$ -receptor antagonist drugs. Our study introduces the  $\alpha_2$ -receptor antagonists as body weight reducing, glucose restoring and insulin and leptin increasing drugs.

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**Key words:** Diabetic rats, leptin, yohimbine, insulin,  $\alpha_2$ -adrenoceptor, streptozotocin

## INTRODUCTION

Leptin is the product of *ob* gene and secreted from adipose tissue (1). Insulin increases plasma leptin levels in normal and diabetic rats (2, 3) and similarly in humans (4). Circulating leptin concentrations correlate with adiposity in both humans (5) and rodents (6, 7). Leptin acts as a signal from peripheral adipose stores to the paraventricular nucleus (PVN) of hypothalamus to decrease food

intake, increase energy expenditure and limit adiposity (8, 2). The  $\alpha_2$ -adrenoceptors ( $\alpha_2$ -ARs) are intrinsic membrane glycoproteins that mediate a variety of important responses. They mediate a variety of functions and have been of major interest for many years as targets for appetite and food intake.  $\alpha_2$ -ARs are present in PVN and stimulation of these receptors at this site increase food intake in rats (9, 10) while antagonism of these receptors have opposite effects (10-12).  $\alpha_2$ -ARs and leptin receptors are found together in many other sites such as cells of Langerhans (13) and adipose tissue. In adipose tissue activation of  $\alpha_2$ -ARs inhibits lipolysis and antagonism of  $\alpha_2$ -ARs has inverse action (14). In the cells of pancreas, inhibition of  $\alpha_2$ -ARs increases insulin secretion (13). Drop in blood concentration

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of glucose may act as a stimulatory signal to  $\alpha_2$ -ARs and results in increased food intake, especially carbohydrates, and decrease in insulin secretion (12, 15). Furthermore, in diabetic animals there is significant reduction of  $\alpha_2$ -ARs (16).

It seems that  $\alpha_2$ -ARs and leptin both contribute to regulation of body weight and study of effect of stimulation or inhibition of  $\alpha_2$ -ARs on plasma levels of leptin can be important for determination of interactions between them in body weight regulation. In this study, we examined the effect of yohimbine as an  $\alpha_2$ -AR antagonist on body weight and plasma leptin, insulin and glucose levels in streptozotocin-induced diabetic and normal rats.

## MATERIALS AND METHODS

Male Sprague Dawley rats were used for this study and all were fed with standard rat chow and housed according to National Institutes of Health guidelines. All animals were allowed free access to food and water. Two types of studies were performed; experiment 1 examined the effects of yohimbine treatment on body weight and plasma levels of leptin, insulin and glucose in normal rats. Experiment 2 consisted of two groups: 1) diabetic rats treated with insulin but not treated with yohimbine, 2) diabetic rats treated with both insulin and yohimbine. We induced diabetes in rats by streptozotocin (STZ) injection (i.p. injection, single dose, 50 mg/kg) to eliminate the insulin increasing effect of yohimbine because STZ destroys cells of Langerhans. Diabetes in rats was confirmed by high blood glucose levels. After induction of diabetes, insulin (NPH) therapy (10 u/kg/day) was initiated for prevention of  $\alpha_2$ -ARs reduction that occurs in diabetes. Body weight and plasma levels of leptin, insulin and glucose were measured in all groups.

### Experiment 1

Five male rats with mean body weight of  $283.6 \pm 13.46$  grams were housed for 8 days. On the third day, yohimbine treatment (2 mg/kg/day) orally was initiated at 10 AM and continued until day 8. One blood sample was collected on day 1 before treatment and three other samples collected on days 4, 6 and 8 after treatment at 12 MD from eye angle.

Plasma was separated after centrifugation of blood samples at 3000 rpm for 5 minutes and frozen at  $-70^\circ\text{C}$  until analysis. Rats were killed on day 8 after blood sampling.

### Experiment 2

Ten male rats with initial body weight of  $295.9 \pm 16.89$  grams were housed for 8 days. Diabetes was induced in rats by an intra-peritoneal injection of 50 mg/kg streptozotocin (Pharmacia and Upjohn co, Kalamazoo, Michigan, USA) on day 2. Diabetes was diagnosed in day 4, defined as blood glucose greater than 350 mg/dl using an Accu-Chek meter (Boehringer Mannheim, Indianapolis, IN) on one drop of blood obtained by tail vein puncture. On the same day, rats were divided in 2 groups each consisted of 5 rats and then medications initiated in two groups: 1) insulin injected subcutaneously (10 u/kg/day), 2) yohimbine used orally (2 mg/kg/day) and insulin injected subcutaneously (10 u/kg/day). All medications were accomplished at 10 AM. One blood sample was collected on day 1 before STZ injection and 3 samples were collected on days 4, 6 and 8 at 12 MD. Plasma was separated after centrifugation of blood samples at 3000 rpm for 5 minutes and frozen at  $-70^\circ\text{C}$  until analysis.

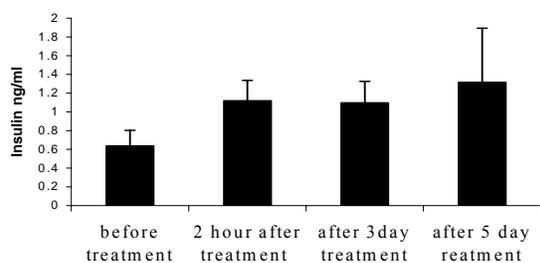
### Measurements

Plasma leptin was assayed using the IBL rat leptin EIA kit (Fujioka, Gunma, Japan). Plasma insulin was measured using the DRG rat insulin EIA kit (Germany). Plasma glucose was measured using the glucose oxidase method with a glucose analyzer 2700 (yellow springs instruments).

Statistical analysis of data was performed with paired-samples *t* test and independent-samples *t* test. *P* values less than 0.05 were assumed significant. Values are defined as mean  $\pm$  standard deviation.

## RESULTS

In experiment 1 plasma insulin level before yohimbine treatment was  $0.63 \pm 0.17$  ng/ml and raised to  $1.12 \pm 0.023$  in first,  $1.09 \pm 0.24$  in second and  $1.32 \pm 0.57$  ng/ml in third sample and these increments were statistically significant ( $P < 0.004$ ,  $P < 0.007$  and  $P < 0.042$ , respectively) (Fig. 1). Leptin

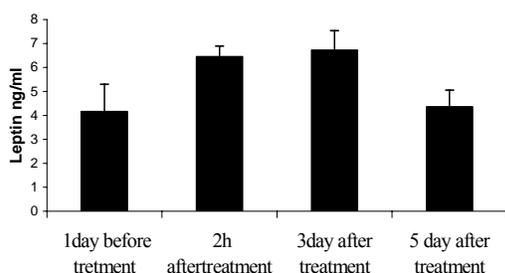


**Fig. 1.** Plasma insulin levels (ng/ml) in normal rats before and after treatment with yohimbine.

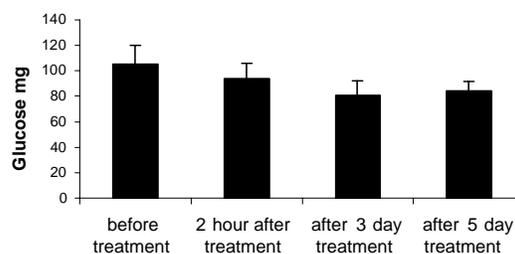
increased in all samples after treatment but these rises were only statistically significant in first and second samples ( $P < 0.005$  and  $P < 0.037$ , respectively) (Fig. 2). Glucose concentration was  $105.2 \pm 14.82$  mg/dl before treatment and diminished to  $94 \pm 12.08$  in first sample,  $80.8 \pm 11.2$  in second sample and  $84.2 \pm 7.46$  mg/dl in third sample after treatment. Decrease was statistically significant only in the second sample ( $P < 0.023$ ) (Fig. 3).

In experiment 2 insulin levels were similar in 2 groups (Fig. 4) but leptin levels were lower significantly in yohimbine treated compared with non-treated rats ( $P < 0.002$ ) (Fig. 5). Also, glucose levels in yohimbine treated rats were significantly lower than non treated rats ( $P < 0.02$ ) (Fig. 6).

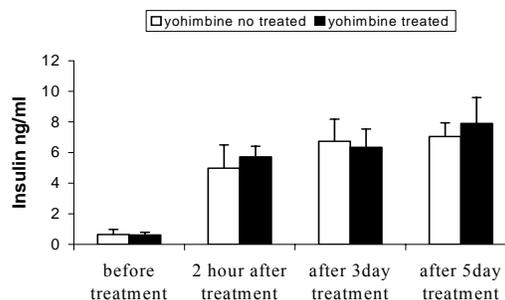
Body weight reduced significantly in yohimbine treated rats in experiment 1 as decreased from  $295.4 \pm 12.3$  grams before treatment to  $247 \pm 12.98$  grams in day 8 ( $P < 0.05$ ). Body weight also diminished from  $288.8 \pm 6.53$  grams before treatment to  $223.8 \pm 6.91$  grams at day 8 in experiment 2 of yohimbine treated rats ( $P < 0.05$ ). Also there was significant reduction between groups in experiment 2 on days 5, 6, 7 and 8 ( $P < 0.038$ ,  $P < 0.01$ ,  $P < 0.002$  and  $P < 0.001$ , respectively) (Table 1).



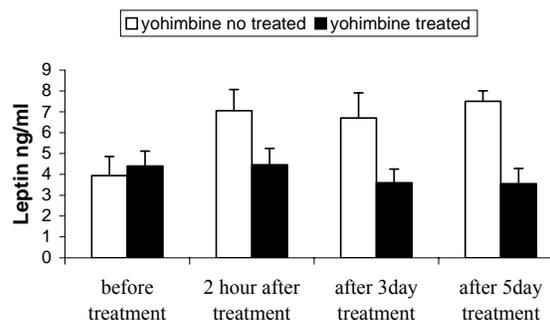
**Fig. 2.** Plasma leptin levels (ng/ml) in normal rats before and after treatment with yohimbine.



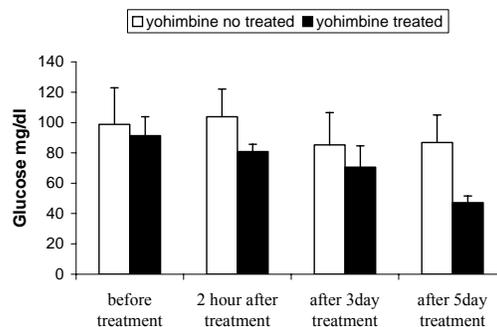
**Fig. 3.** Plasma glucose levels (mg/ml) in normal rats before and after treatment with yohimbine.



**Fig. 4.** Plasma insulin levels (ng/ml) in yohimbine treated and non-treated diabetic rats before and after treatment with insulin subcutaneously (10 u/kg/day).



**Fig. 5.** Plasma leptin levels (ng/ml) in yohimbine treated and non-treated diabetic rats before and after treatment with insulin subcutaneously (10 u/kg/day).



**Fig. 6.** Plasma glucose levels (ng/ml) in yohimbine treated and non-treated diabetic rats before and after treatment with insulin subcutaneously (10 u/kg/day).

**Table 1.** Comparison of body weight for 8 days in normal yohimbine treated (n=5), STZ-induced diabetic rats treated with insulin (n=5) and STZ-induced diabetic rats treated with both insulin and yohimbine (n=5)\*

Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Normal-treated	283.6 ± 13.46	290.4 ± 11.2	295.4 ± 12.3	287 ± 11.11†	268.4 ± 11.52†	261.2 ± 12.99†	253.4 ± 14.93†	247 ± 12.98†
Diabetic-non treated	303 ± 21.76	307 ± 22.53	287.8 ± 21.36†	269.8 ± 21.16†	269 ± 20.11	268.8 ± 18.95	270.2 ± 17.81	267.8 ± 19.1
Diabetic-treated	288.8 ± 6.53	294.4 ± 5.73	276.6 ± 5.5†	257.2 ± 5.5†	245 ± 8.09†	238 ± 8†	230.8 ± 6.53†	223.8 ± 6.91†

Abbreviation: STZ, streptozotocin.

\*Data are expressed as mean ± standard deviation.

†P < 0.05. Statistical comparisons with controls were carried out using unpaired Student's t test.

## DISCUSSION

In this study we found that antagonism of  $\alpha_2$ -ARs by yohimbine increased plasma leptin levels in normal rats. This increase is not seen in STZ-induced diabetic rats that were treated with yohimbine. Therefore this rise may have resulted from insulin increasing effect of this drug.

Animal and *in vitro* studies have provided clear evidence that postsynaptic  $\alpha_2$ -ARs are present on pancreatic cells (17) and their selective stimulation inhibits glucose-induced insulin release (18). In humans, the pioneer studies of Robertson and Porte showed that in the post absorptive state phentolamine, an  $\alpha$ -adrenoceptor antagonist, increased while propranolol, a non selective  $\beta$ -blocker, inhibited insulin release (19). In addition, phentolamine potentiated while propranolol depressed insulin release in response to epinephrine-induced hyperglycemia.

Normal subjects and diabetic patients were found to have improved insulin secretion in response to intravenous glucose pulses when  $\alpha$ -adrenoceptors were blocked with phentolamine (20). Lacy and Chan reported that expression of  $\alpha_2$ - and  $\beta$ -adrenoceptor subtypes occur in human islets of Langerhans (21). It has been reported that, in conscious dogs, the ingestion of deriglidole ( $\alpha_2$ -ARs antagonist) results in a prompt (within 30 minutes) increase in plasma insulin (22). However, some studies in humans did not show this insulin increasing effect of  $\alpha_2$ -ARs blockade (23, 24), albeit the glucose decreasing effect was observed (24).

Other studies in humans indicated that exercise-induced decrease in insulin secretion was mediated via  $\alpha_2$ -ARs and was abolished by blockade of these receptors by yohimbine (25).

In this study we found that yohimbine increased plasma insulin and declined glucose in normal rats. In diabetic rats treated with insulin (10 u/kg/day) and yohimbine, plasma glucose levels were markedly lower than diabetic rats just treated with insulin (10 u/kg/day). We suggest that enhancement of plasma leptin was associated with insulin increasing effect of yohimbine. Body weight reducing effect of  $\alpha_2$ -ARs antagonist drugs has been reported in many studies in rats (9, 11, 12, 26) and in dogs (27). Sprague Dawley rats exhibit a relatively homogeneous pattern of daily weight gain when fed only with laboratory chow (28). In this study, we observed significant reduction of body weight in normal rats. We observed that rats lost their weight day by day after yohimbine treatment. This body weight loss consisted of two parts; first pause of weight gain (about 5-7 grams per day) and second decrement of initial weight (about 5-7 grams per day), together resulted in a 10-14 grams weight loss per day, indicating that yohimbine potently reduce body weight in rats.

We suppose a new hypothesis to explain this action of  $\alpha_2$ -ARs antagonist drug in normal rats. This hypothesis indicates leptin increment after  $\alpha_2$ -ARs antagonist medication may have contributed to body weight-reducing effect of this drug. Leptin increases lipolysis by activation of sympathetic nervous system especially  $\beta$ -adrenergic receptors in adipose tissue in the manner like  $\alpha_2$ -ARs antagonist drugs

and decreases appetite by acting on hypothalamus and these actions tend to reduce body weight (8). Also we observed significant reduction of body weight in diabetic yohimbine treated rats. As indicated in results, leptin was significantly lower in STZ-induced diabetic yohimbine treated rats compared with non treated STZ-induced diabetic rats. There are many reports indicating  $\alpha_2$ -ARs antagonist drugs reduced appetite by affecting the hypothalamus (9-12). We assume that direct effect of yohimbine on hypothalamus in diabetic rats is responsible for body weight reduction. In normal and diabetic rats reduction of appetite by the direct effect of yohimbine on hypothalamus may be the common reason for the weight decrement. But effect of yohimbine on reduction of leptin and body weight in diabetic rats needs further investigation (29). There are a few studies about this action of  $\alpha_2$ -ARs antagonist drugs in humans. Titouamane and Baudouin reported that brimonidine a selective  $\alpha_2$ -ARs agonist reduces appetite in human subjects (30).

In conclusion,  $\alpha_2$ -ARs antagonist yohimbine reduced body weight in normal and STZ induced diabetic rats. Leptin increased after yohimbine treatment in normal rats while decreased in STZ-induced diabetic rats. This plasma leptin increase attributed to insulin increasing effect of  $\alpha_2$ -ARs antagonist drugs. This plasma leptin increase in normal rats may be contributed to the weight reducing effect of  $\alpha_2$ -ARs antagonist drugs. Our study introduces the  $\alpha_2$ -ARs antagonists as body weight reducing, glucose restoring and insulin and leptin increasing drugs. We suggest that further studies should be done in future to confirm that  $\alpha_2$ -ARs may act in regulation of body weight in humans and may be used as an anti-obesity drug.

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### Conflict of interests

The authors declare that they have no competing interests.

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