Gender Differences in Nicotine Induced Dyslipidemia and Hyperglycemia in Mice

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Abstract: The purpose of this study is to examine the prevalence of metabolic syndrome in nicotine treated male and female mice and to evaluate gender related differences. For these purposes adult male and female BALB/C mice were subjected to chronic nicotine treatment (3.08mg/100ml in drinking water) for 4 weeks. Serum glucose, albumin, corticosterone and lipid profile levels were determined. Body weight changes were also monitored. We have found that nicotine treatment raises total cholesterol and glucose levels more in male as compared to female mice. Low density lipoprotein cholesterol (LDL-C) levels were increased by 35% ($P<0.01$) only in male mice. However rise in triglycerides were greater in females (28%) than males (21%) when compared with their respective controls. Serum albumin levels were increased in both sexes showing 13% greater increase in males as compared to females. However nicotine treatment had no effect on high density lipoprotein cholesterol, corticosterone levels and body weights in both genders. It is concluded that nicotine use is positively associated with LDL-C in males; the results are discussed in relation to prevalence of metabolic syndrome and risk of cardiovascular events in nicotine users.

Keywords: Cholesterol, lipid profile, metabolic syndrome, corticosterone, nicotine, mice.

INTRODUCTION

Nicotine is one of the key psychoactive ingredients in tobacco that contribute to the harmful tobacco smoking habit leading to high morbidity and mortality all over the world .Smoking is broadly accepted as a major risk factor for cardiovascular diseases [1]. Previous studies have shown that smoking reduces insulin sensitivity, induces insulin resistance [2, 3] and enhances cardiovascular risk factors, such as elevated plasma triglycerides [4] reduced high density lipoprotein cholesterol (HDL-C) [5] and hyperglycemia [2, 6]. Number of studies has shown that smoking is associated with metabolic abnormalities and metabolic syndrome [7, 8]. Oh *et al*. [9] reported that chronic smoking is associated with higher triglycerides and lower HDL cholesterol with a dose effect; however, other key components of the metabolic syndrome, such as hypertension and hyperglycemia, were less common in smokers. It has been shown that oral administration of nicotine raises plasma total cholesterol and low density lipoprotein cholesterol (LDL-C), and lowers HDL-C in normal dietary condition [10].

Studies have shown that nicotine have marked effects on endocrine function. The relationship between nicotine and cortisol is important for at least three reasons. First, the hypothalamic pituitary adrenal axis implicated in addictive processes. Second, increased levels of cortisol have a variety of adverse effects on biological processes, including lipid profiles, immune function, central adiposity, bone mineral density and reproductive function [11]. Cortisol may therefore arbitrate some of the effects of smoking on health consequences such as cardiovascular disease and the metabolic syndrome. Third, cortisol is highly sensitive to psychological stress. Smoking cessation is stressful for many smokers, for this reason they fail in quit attempts. It has been proposed that cortisol is directly involved in this process, and that changes in cortisol following smoking cessation may envisage early relapse [12].

Previously we have reported that chronic nicotine treatment fails to alleviate depression like symptoms, worsen lipid profile and glucose homeostasis in forced swim mice that can be related to increased risk of cardiovascular illness and depression among chronic smokers particularly living in stressful situations [13]. The present study is designed to examine the occurrence of metabolic syndrome in nicotine treated male and female mice and to evaluate gender related differences.

MATERIALS AND METHODS

Animals and Treatments

All animal procedures described below were conducted in strict accordance with the national research council for the care and use of laboratory animals (1996). Ethical approval was obtained from

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institutional animal ethics committee, University of Karachi. All efforts were made to minimize the number of animals and any pain/distress they might incur. Adult male and female BALB/C mice weighing 25-30 gm were housed in 6 per cage under light and dark conditions at 25 ± 20 ºC and maintained at lab chow and water *ad libitum* under standard housing conditions. Mice were divided into 2 groups (male and female). Each group had 12 mice. Those assigned as drug treated (n=6) were given nicotine hydrogen tartrate (3.08 mg/ml) in 100 ml of drinking water for 21 days while the control group received drinking tap water. After last treatment animals were decapitated. Blood was collected and centrifuged at 4000 rpm for 30 minutes. The serum was separated as supernatant and frozen at –20 ºC until analysis.

Analysis of Serum Parameters

Serum glucose concentrations were determined by O-toluidine method [14]. Serum albumin concentrations were determined by dye-binding method [15]. Serum cholesterol, triglycerides, HDL-C and LDL-C were estimated by using kit method (Randox®, Private Ltd). Serum corticosterone levels were determined by Glick *et al*. [16].

Chemicals and Drugs

Nicotine Hydrogen (+)-tartrate was purchased from Sigma chemical Co. All other chemicals were of analytical grade.

Statistical Analysis

The data was analyzed either by two-way ANOVA [factor 1= sex, factor 2 = drug and interaction (sex and drug) between the two factors]. Individual comparison was made by using Newman-keuls q-test or student's *t*-test where appropriate. Difference between groups were considered significant when P<0.05.

RESULTS

Table **1** shows the effects of chronic administration of nicotine on lipid profile and serum corticosterone levels in male and female mice. Data analyzed by Two-way ANOVA which shows effect of sex was significant on total cholesterol, HDL-C, LDL-C and triglycerides F =31.00 (P<0.01), F = 7.71 (P<0.05), F= 20.02 (P<0.01) and F = 16.28 (P<0.01) respectively. Effect of drug was significant on total cholesterol F = 18.01 (P<0.01), LDL-C F = 12.78 (P<0.01), triglycerides F= 15.99 (P<0.01) and corticosterone F=7.46 (P<0.01) while effects on HDL-C remained non-significant. The interaction between the two (sex x drug) was not significant. Effect of drug was significant on corticosterone levels (F= 7.46; P<0.01).

Figure **1** shows the effects of chronic administration of nicotine on glucose concentration in mice. Data analyzed by Two-way ANOVA followed by Newman-keuls q-test. The results show that the effects of sex were significant on glucose concentration F=154.12 (p<0.01). However the interaction between the two (Sex X Drug) was not significant.

Figure **2** shows the effects of chronic administration of nicotine on body weight in male and female mice. Data was analyzed by Two-way ANOVA followed by Newman-keuls q-test. The results show that the effect of drug was significant F=47.96 (P<0.01) while the

Table 1: Effect of Chronic Nicotine Administration in Male and Female Mice on Serum Lipid Profile and Corticosterone

PARAMETERS	MALE		FEMALE		Two-Way ANOVA Df 1,20		
	Control	Drug	Control	Drug	Sex	Drug	Sex X Drug
Total Cholesterol (mg/dl)	163.9 ±13.01	192.3 ±14.45**	154.2 ±12.06†	177.8 ±13.9**†	F=31.00 P<0.01	F=18.01 P<0.01	F=0.71 N.S
HDL-Cholesterol (mg/dl)	36.5 ±2.95	32.52 ±1.58	38.7 ±2.31	33.4 ±2.59	F=7.71 P<0.05	F=0.86 N.S	F=0.13 N.S
LDL-Cholesterol (mg/dl)	49.2 ±4.43	66.8 ±3.01**	41.05 ±3.64	52.08 ±4.3††	F=20.02 P<0.01	F=12.78 P<0.01	F=1.06 N.S
Triglycerides (mg/dl)	70.6 ±2.26	85.5 ±3.53**	62.4 ±2.59††	79.5 ±1.95**	F=16.28 P<0.01	F=15.99 P<0.01	F=3.40 N.S
Corticosterone (µg/dl)	65.30±3.8	58.6±3.13	74.1±3.6	71.8±4.03	F= 1.22 NS	F=7.46 P<0.01	F=0.299 NS

Experimental details are given in materials and methods section. Values are mean ± SEM for n=6 mice. Statistical analysis was performed using two-way ANOVA followed by Newman-Keuls q-test. The significance of the difference is indicated by ** P<0.01 from respective controls and † P<0.05 and †† P<0.01from similarly treated male mice.

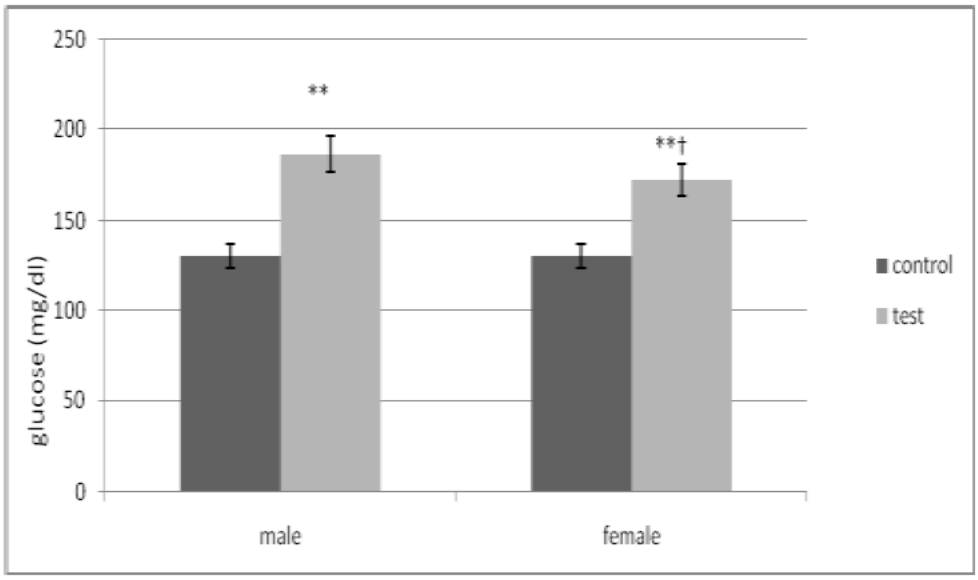

Figure 1: Experimental details are given in materials and methods section. Values are mean ± SEM for n=6 mice. Statistical analysis was performed using two-way ANOVA followed by Newmankeuls q- test. The significance of the difference is indicated by **P<0.01 from respective controls and † P<0.05 from similarly treated male mice.

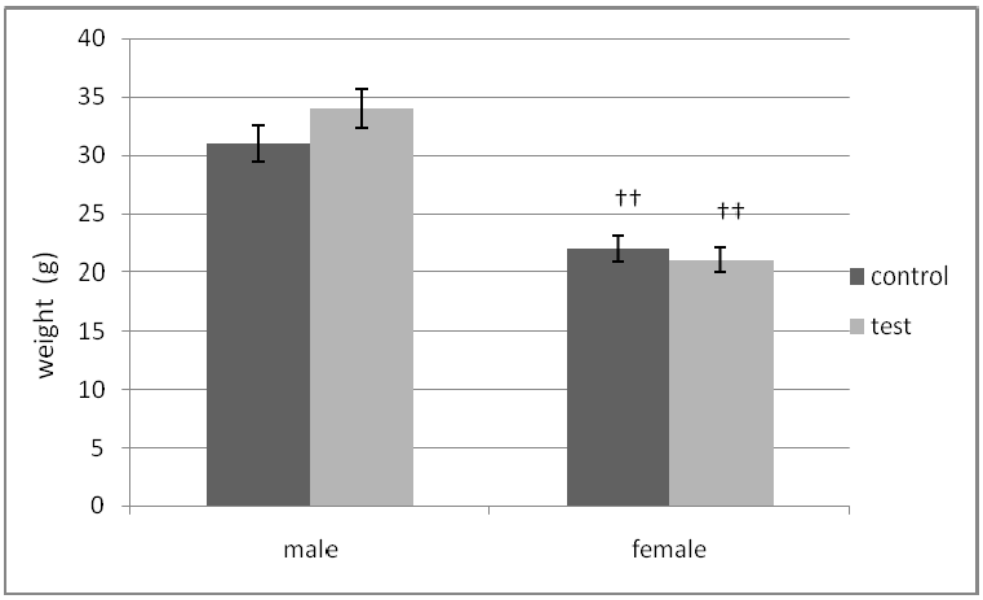

Figure 2: Experimental details are given in materials and methods section. Values are mean ± SEM for n=6 mice. Statistical analysis was performed using two-way ANOVA followed by Newman-keuls q- test. The significance of the difference is indicated by †† P<0.01 from similarly treated male mice.

effect of sex and sex X drug interaction was not significant.

Figure **3** shows the effects of chronic administration of nicotine on serum albumin concentration in male and female mice. Data analyzed by Two -way ANOVA followed by Newman-keuls q-test. The results show that the effect of sex and sex x drug was not significant. In contrast the effect of drug was significant F= 115.7 (P<0.01).

DISCUSSION

Present study shows that treatment of nicotine (3.08 mg/kg/day) for 4 weeks reduced body weight by 43% in females, but had no effect in the male mice (Figure **2**). We have also found that nicotine treatment increased total cholesterol, triglycerides and LDL-C in both male and female mice. However Serum HDL-C remained unaltered in both genders (Table **1**). These findings are in agreement with previous finding by Bibi *et al.*, [13].

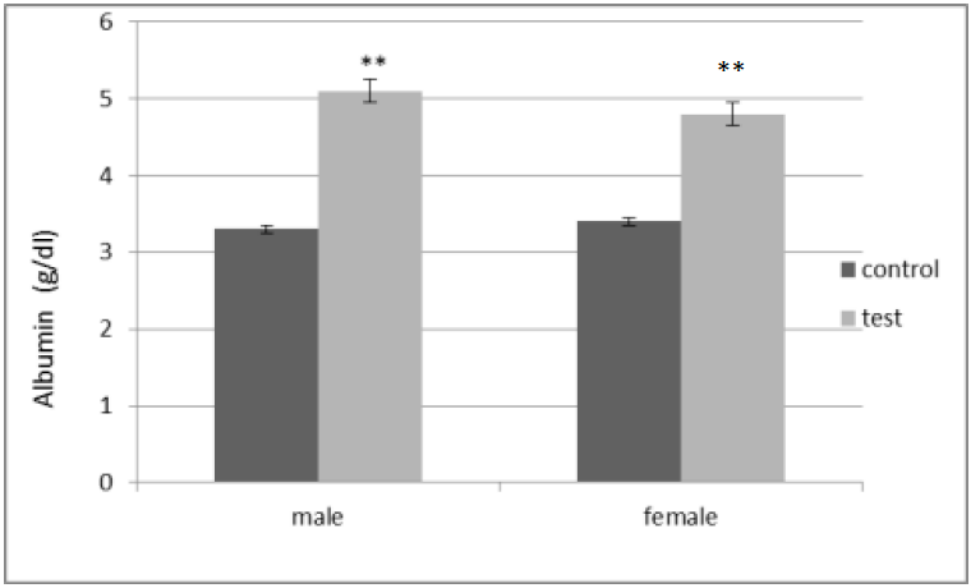

Figure 3: Experimental details are given in materials and methods section. Values are mean ± SEM for n=6 mice. Statistical analysis was performed using two-way ANOVA followed by Newman-Keuls q-test. The significance of the difference is indicated by **P<0.01 from respective controls.

The increased glucose level following nicotine treatment is associated with the activation of enzyme HMG CoA reductase, it is the rate controlling enzyme of metabolic pathway of cholesterol synthesis. These findings are consistent with those reported earlier [17, 18]. Smoking appears to have at least two lipid effects that may promote coronary heart disease (CHD) and atherosclerosis: increased plasma free fatty acids and decreased plasma HDL-C fraction [19]. Nicotine, by release of catechol amines, induces lipolysis and releases plasma free fatty acids. There is evidence that these free fatty acids are primarily taken up by the liver, which might be expected to increase the synthesis of very low density lipoproteins [20] consistent with changes described in cigarette smokers [21]. Studies on the effects of nicotine on lipids in animals are conflicting. Injection of nicotine or feeding of nicotine has been reported [22] to increase total cholesterol in rabbits and monkeys receiving a high cholesterol diet. Later it was reported [17] that the activity of lipoprotein lipase in extra hepatic tissues and plasma lecithin cholesterol acyl transferase activity were significantly lower in nicotine-treated rats. These results indicate that nicotine exerts hyperlipidemia effects particularly by increasing the synthesis and secretion of triglyceride-rich lipoproteins. In adult female rats, nicotine treatment increases total cholesterol and non-esterified fatty acids [23]. Present study clearly shows a strong relationship between elevation of serum lipids and nicotine use in both males and females. The increase in serum total cholesterol, LDL-C in males assumes a great significance since this has been the pattern associated with CHD.

Nicotine has been found in some human studies to transiently elevate blood glucose levels. Chronic nicotine administration is accompanied by significant decreases in circulating insulin levels. Nicotine increases levels of catecholamines, but this effect is short-lived [24]. The ingestion of nicotine results in a discharge of epinephrine from the adrenal cortex. This causes a sudden release of glucose. It has been reported that nicotine releases acetylcholine from the cholinergic synaptic vesicles effectively and increases the removal of glucose from the liver indirectly through stimulating the muscarinic receptors in the nervous system leading to the stimulation of insulin secretion from pancreas [25]. We have found increase in glucose, albumin (Figures **1**, **2**) and triglyceride levels (Table **1**) in both male and female mice. The triglyceride/high-density lipoprotein abnormalities have recently been suggested to be related to insulin resistance. In fact, it has been proposed that insulin resistance is a potential key link between cigarette smoking and cardiovascular disease [26].

Acute administration of nicotine activates the hypothalamic-pituitary-adrenal axis resulting in increased plasma corticosterone in both male and female adult and adolescent rats, effects that are more pronounced in adult females compared to adult males [27- 29]. Experimental data from rats show that nicotine does not act directly at the hypothalamic

paraventricular nucleus, the site of the corticotrophin-releasing factor neurons crucial to the regulation of adrenocorticotropic hormone (ACTH). However, brainstem catecholaminergic regions projecting to the paraventricular nucleus showed a regionally selective and dose-dependent sensitivity to nicotine, particularly the noradrenergic/adrenergic nucleus tractus solitarius [30]. Catecholamine release in response to nicotine, which could then affect pituitary secretion directly, has been demonstrated both *in vitro* [31] and *in vivo* in animals [32-33]. Therefore, in animals, it is brainstem catecholaminergic neurons that play a role in the central effect of nicotine on ACTH secretion. Additionally, it is possible that neurotransmitters other than noradrenaline may be involved in the acute ACTH response to systemic nicotine [30].We have found that chronic administration of nicotine had no effect on plasma corticosterone concentration in both male and female mice (Table **1**). Earlier it has also been reported [31] that ACTH levels are not altered in chronic smokers in contrast to the acute effect of smoking. This is probably due to desensitization of the central nicotinic cholinergic receptors involved [34].

CONCLUSION

We have found that among the features of the metabolic syndrome, only dyslipidemia is associated with chronic nicotine administration. Nicotine also causes moderate increase in blood glucose levels, may be due to nicotine induced release of adrenaline, leading to increase production of glucose by the liver. Both male and female are vulnerable to metabolic syndrome when exposed to nicotine. It could be inferred that nicotine use increases LDL-C in males making them more susceptible to cardiovascular event as compared to females. It is concluded that gender differences in nicotine's effects are important factors to consider in developing better prevention and treatment strategies in smoking cessation.

CONFLICTS OF INTEREST

There was no conflict of interest.

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