The effect of long term nicotine exposure on nicotine addiction and fetal growth

Uzun süreli nikotine maruziyetin nikotin bağımlılığına ve fetüsün büyümesine etkisi

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Abstract

Objective: To investigate the effect of nicotine exposure starting before coitus and continuing during pregnancy and lactation period on delivery rate, fetal growth and nicotine addiction in rats.

Material and Methods: Ten female Swiss Albino rats were divided into 2 groups as the nicotine group (NG) (n=5), and the control group (n=5), conceived by adding 2 male rats to each group. While the control group was given only normal drinking water, 0.4 mg/kg body weight (BW)/day nicotine was given to the NG in drinking water. After delivery, the BWs of pups were recorded weekly for 6 weeks and their drinking water preferences were assessed. Meanwhile, pups of the NG continued to receive 0.4 mg/kg/day nicotine for 12 months while the controls continued with normal drinking water.

Results: At the end of the 6th week, it was determined that 30 (69%) rats out of 43 in the NG and only 7 rats (20%) out of 35 in the control group preferred the nicotine added drinking water ($p<0.05$). No significant difference was observed between control and NGs in postnatal birth weights and BWs recorded for 6 weeks. On the contrary, a significant decrease ($p< 0.05$) was observed in the BWs of NG at the end of 12 months nicotine exposure.

Conclusion: Use of maternal nicotine in pregnancy and lactation periods, even at a low dose, may be effective in nicotine addiction development although it may not affect delivery rate, and BWs of pups after delivery and during six weeks follow up in the lactation period.

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Key words: Nicotine, pregnancy, nicotine addiction, fetal growth, delivery rate

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Özet

Amaç: Sıçanlarda koitusdan önce başlayıp gebelik boyunca ve laktasyon döneminde devam eden nikotin maruziyetinin doğum oranı, fetal büyüme ve nikotin bağımlılığına olan etkilerinin araştırılması

Gereç ve Yöntemler: On dişi Swiss Albino sıçan kontrol grubu (n=5) ve nikotin grubu (n=5) olarak iki gruba ayrıldı ve aralarına 2 erkek sıçan eklenerek gebe kalmaları sağlandı. Kontrol grubuna normal içme suyu verilirken, nikotin grubuna içme suyu içerisinde 0.4 mg/kg vücut ağırlığı/gün nikotin eklendi. Doğumdan sonra yavru ratların vücut ağırlıkları, 6 hafta boyunca haftalık kaydedildi ve su tercihleri değerlendirildi. Bu arada nikotin grubunun yavru sıçanları 0.4 mg/kg/gün nikotin 12 ay boyunca devam ederken, kontroller normal içme suyuna devam etti.

Bulgular: Altı hafta sonunda nikotin grubundaki 43 sıçanın 30'u (%69) ve kontrol grubundaki 35 sıçanın 7'si (%20) nikotin eklenmiş suyu tercih etti ($p<0.05$). İki grup arasında doğumda ve 6 hafta boyunca kaydedilen vücut ağırlıkları arasında herhangi bir farklılık saptanmazken, 12 aylık nikotin maruziyeti sonunda nikotin grubunun vücut ağırlığı istatistiki olarak anlamlı düşük bulunmuştur ($p<0.05$).

Sonuçlar: Nikotin, gebelik ve laktasyon dönemlerinde düşük dozda dahi kullanımı nikotin bağımlılığının gelişmesine etkili iken; doğum oranı ve yavru ratların doğumda ve 6 haftalık takibinde vücut ağırlıkları üzerine bir etkisi izlenmemiştir.

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Anahtar kelimeler: Nikotin, gebelik, fetal büyüme, doğum oranı, nikotin bağımlılığı

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Introduction

Cigarette smoking is defined as a bio-socio-psychological state of intoxication by the World Health Organization (WHO). Cigarette smoking is a quite common habit worldwide and 90% of smokers start this habit before the age of 20. Interestingly, since the number of female smokers is increasing daily, this leads to more frequent encounters with pregnant smokers (1). During the last two decades, smoking among pregnant women in the developed countries decreased by about 60-75% (2). 20-25% of pregnant women in South America and 30-36% of pregnant women in Spain smoke and approximately 41% of them attempt to give up this habit every year. However, only 10% of them succeed in quitting smoking. Nicotine replacement therapies (NRT) have been developed for nicotine addiction so as to boost this achievement (3).

There are more than 4000 chemical compounds in the cigarette. Some of them are carcinogenic substances and the most hazardous ones are arsenic, benzene, cadmium, hydrogen cyanide, toluene, ammonia and propylene glycol (4). Although it is not known for certain which of these chemicals

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are harmful for the fetus, it is believed that nicotine especially may affect the pregnancy outcomes negatively (5).
Cotinine, the metabolite of nicotine, passes the placental barrier, which has been proven by showing it both in the amniotic fluid and fetal cord blood (6). Nicotine has been proposed to have negative effects on the fetus but it has not been explained exactly by which mechanism this situation may occur. Cheryl A. et al. (7) suggested that the direct toxic effect of nicotine on the fetus may be attributed to a decreased oxygen amount as a result of vasoconstriction in uterine arteries. The most-studied complication of smoking during pregnancy has been low birth weight (< 2500) (8,9). It was demonstrated that, in female smokers, the risk of having babies with low birth weight increases by 1.5-3.5 fold and that this risk increase is correlated with the increase in cigarette consumption ratio (10).
The nicotine dose in one cigarette is not life-threatening, but it has an addictive effect, which makes smokers keep on smoking and inhaling the other chemicals in tobacco, causing the health risks (11). In cigarette smoking, nicotine is quickly absorbed into the blood circulation, reaching the brain in 10 seconds, much quicker than other tobacco products. This is one of the major reasons why cigarette smoking has a high potential of having an addictive behaviour (12). Nicotinic acetylcholine receptors (nAChRs) are concentrated particularly in the areas of cognitive function such as the prefrontal cortex, basal ganglia, nucleus ceruleus and in the mesolimbic area. Nicotine activates the nAChRs and activation of these reseptors not only leads to release of acetylcholine, dopamine and glutamate but also modulates the other neurotransmitters such as noradrenaline and serotonin (13).
In this study, our aim is to investigate the effect of nicotine exposure starting before coitus and continuing during pregnancy and lactation period on fetal growth and nicotine addiction and to assess the effect of long term nicotine exposure on the physiological development of the pups by measuring the BWs of rats at the end of 12 months of nicotine exposure.

Material and Methods

In this study, approved by the Animal Ethics Committee of Ege University School of Medicine, Bornova, İzmir, Turkey,10 female and 4 male Swiss-Albino rats (200±50 g) were used. Rats were housed in a temperature-controlled room with a 12-hour light/dark cycle. The animals were maintained on standard laboratory animal chow and given water ad libitum. They were maintained in accordance with the guidelines for animal welfare. Female Swiss Albino rats were divided into 2 groups as the nicotine group (n=5), and the control group (n=5). They were conceived by adding 2 male rats to each group. While the control group was given normal drinking water, 0.4 mg/kg body weight (BW)/day nicotine (nicotine hydrogen tartrate, SIGMA 2.22 mg/kg/BW) was prepared freshly every day and added to the nicotine group's drinking water. After birth, firstly, the BWs and malformations were recorded and the offspring were left in the same cage with their mothers and breast-fed. During this lactation period, daily water consumption and body weights were recorded every week.
Pups, which had been kept beside their mothers as they breast-fed in the first six weeks following the birth were separated from their mothers at the end of 6th week and put individually in different cages in order to detect nicotine preference. Their preference for normal or nicotine added drinking water was determined through the method of "two bottle free choice". Nicotine was offered under the homecage, two-bottle choice regimen between nicotine added water and normal water with unlimited access for 24 h/day. The bottles were refilled every day with a fresh solution and their left–right positions interchanged daily to avoid development of position preference. Saccharin (Huxol sweetener, 2.4 g saccharin/200 mL) was dissolved in nicotine added water so as to mask the bitter taste of the water caused by nicotine, and make it easier for the rats to drink it. The normal water was also sweetened by adding saccharin during the test in order not to influence their water preferences. Daily nicotine was added and normal water consumed in each cage was measured and the water which the pups preferred was noted. This test was performed every day for one week. Then, low dose nicotine administration to pups born in the nicotine group was continued as 0.4 mg/kg/day for 12 months and their BWs were compared to controls at the end of this period.

Statistics

The statistics program of SPSS 13.0 was used in the statistical assessment of findings. Non-parametric tests (Mann Whitney U and Chi Square) were used to compare body weights and nicotine addiction. A $p<0.05$ value was accepted as significant.

Results

Forty-three pups were born in the nicotine group while 35 pups were born in the control group. No congenital malformation was observed in any of these rats.
No significant difference was observed between the pups in control and nicotine groups when the rats were compared in terms of their first measured post-natal birth weights and their weekly BWs recorded weekly for a period of 6 weeks (Table 1, Figure 1).
Pups of both the nicotine and control groups were kept beside their mothers as they continued breastfeeding in the first six weeks following the birth. At the end of the 6th week, they were put individually in separate cages in order to assess nicotine preference and addiction, and their preference for normal or nicotine added drinking water was determined with graduated bowls. Each day for one week, the type of water the pups preferred was recorded. It was determined that 30 (69%) pups out of 43 in the nicotine given group and only 7 pups (20%) out of 35 in the control group preferred the nicotine added water, while the others preferred the nicotine-free water ($p< 0.05$) (Table 2).
At the end of the 12th month, when the final BWs of the control and nicotine groups were measured, it was determined that the final BW of the nicotine group was significantly lower than the final BW of the control group ($p<0.05$) (Figure 2).

Table 1. The follow up of pups' body weights for six weeks

Groups (n=78)	1st day (g)	1st week (g)	2nd week (g)	3rd week (g)	4th week (g)	5th week (g)	6th week (g)
Control Group (n=35)	5.6	10.2	18.7	26.4	37.1	55.9	69.1
Nicotine Group (n=43)	5.7	10.9	19.8	30.4	44.1	61.9	70.0
No significant difference was observed between the pups in control and nicotine groups for a period of six weeks (p>0.05 each week)							

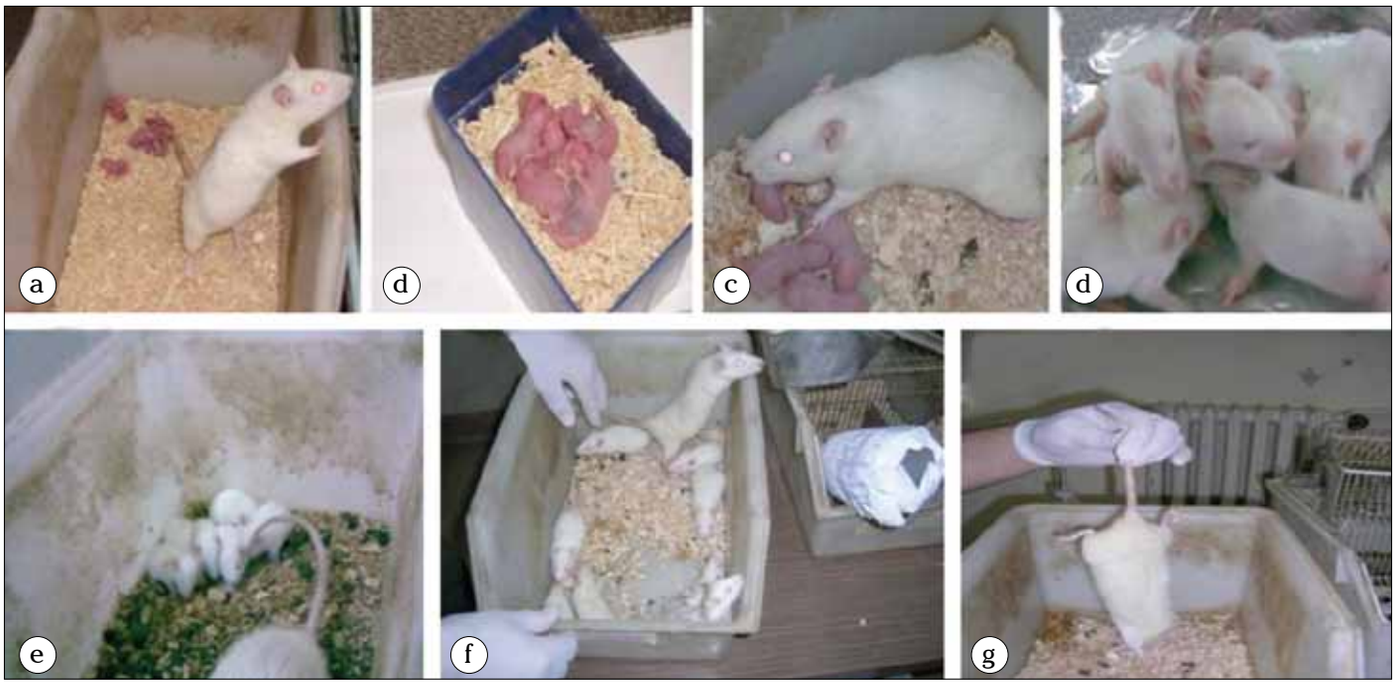

Figure 1. The follow up period of rats for six weeks a) after delivery, b) one week old, c) two weeks old, d) three weeks old, e) four weeks old, f) five weeks old, g) at the end of sixth week

Table 2. Water prefence ratio of rats in two bottle free choice test. Nicotine added water preference ratio was significantly higher in the nicotine group compared to the control group (p< 0.05)

Groups (n=78)	Normal drinking water n (%)	Nicotine-added water n (%)
Nicotine group (n=43)	13 (31%)	30 (69%)
Control group (n=35)	28 (80%)	7 (20%)

Discussion

It was reported that an average of 1.0 mg nicotine is obtained from smoking a cigarette and this intake varies between 0.37 mg and 1.56 mg according to individual differences in its metabolism (14). Nicotine intake via a parenteral method equals many times more nicotine intake when compared to per oral method. For this reason, it would be more accurate to assess the real effects of nicotine by a method mimicking its natural usage such as smoking and nicotine replacement treatment. This is the first study in the literature investigating

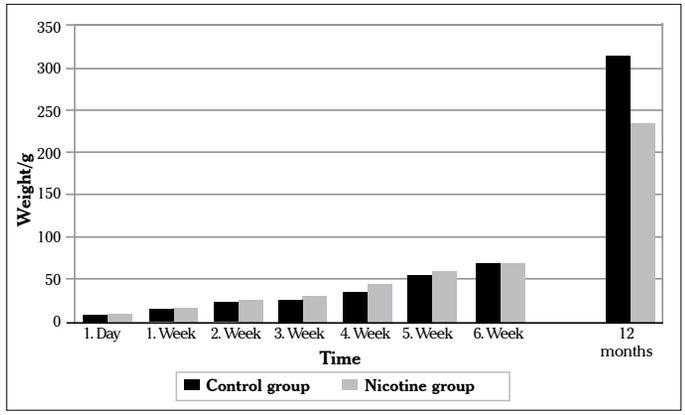

Figure 2. The body weights of the study groups during 12 months follow up

12 months mimicking long-term chronic nicotine exposure. Furthermore, even the studies that are published as chronic nicotine administration lasted 3 months at most (15-17). However, since smoking causes harm to the organism after chronic usage, determining the long term effects of nicotine is required. In the present study, nicotine exposure which started before

coitus and continued during pregnancy and lactation periods was evaluated. In addition, giving low dose nicotine in drinking water to pups of the nicotine group continued for 12 months after a two-bottle free-choice test in order to assess their physiological development by measuring BWs and compared with the BWs of pups in the control group which continued with normal drinking water.

Huang et al. (15) used the oral gastric intubation model and nicotine (6 mg/kg/day) was given in milk-formula for seven postpartum days. At the end of the study, the nicotine group was found to have lower BWs compared to controls. This report can be accepted as an experimental model mimicking chronic nicotine consumption. In addition to this the nicotine dose given to pups with BWs nearly 10-15 g by milk formula corresponds to an amount of at least 10 times higher plasma concentrations of the nicotine dose. For this reason, even this short time of nicotine exposure had an irreversible detrimental effect on pups and their suggestions should not be considered as reliable. In the present study, after conducting the weekly body weight follow-ups of pups, no statistically significant difference was found in final body weights between the nicotine and control groups at birth and during six week follow up. This shows that exposure to low dose nicotine during pregnancy and lactation has no negative effect on the physiological development of new-born pups until the end of the 6th post-natal week. These findings are compatible with other studies that claim that nicotine has no effect on fetal growth (18-20).

The results of the present study are consistent with the studies reporting that short-term nicotine administration has an anorexic effect on animals during the developmental period, especially the puberty period (21, 22). Weight loss induced by nicotine was found to be associated with fat tissue decrease and changes in fat composition (23). It can also be explained by a decrease in food intake desire and increase in energy spending (24, 25). Nicotine controls the food intake and energy spending directly or indirectly via activating the nicotinic acetylcoline receptors (nAchR) and presynaptic receptors in the hypothalamus regulating nutrition and energy metabolism (26). Flyn et al. (27) reported that after oral nicotine administration in different concentrations, “behavior of preference” occurred on the 8th day in adolescent Sprague-Dawley rats, while in another animal study it was stated that no particular nicotine preference occurred on the 12th day of the experiment (28). In the present study, this test was applied after the pups stopped breast-feeding at the end of the 6th postnatal week, and the nicotine preferences of pups were found to differentiate following day 2. It was found that the nicotine added water preference ratio was significantly higher in the nicotine group compared to the control group. The results of this study are very important in terms of showing that using nicotine during pregnancy and lactation period may be effective in developing nicotine addiction in new-borns.

Many studies reported that nicotine has been proved to be a potent pro-oxidant to the spermatozoa population and is able to alter the fertility potential by inducing membrane damage and changing both the sperm morphology and motility in man and by decreasing granulosa cell proliferation and ovarian vascularization but on the contrary increasing ovarian cell apoptosis in females (29, 30). However; in the present study, the rats drinking nicotine-added water became pregnant in a shorter time than controls and delivered more pups having similar body weights in comparison to the pups of the controls . This may be associated with euphoriant effects of nicotine which led to an increase in physical motility and sexual behaviour of rats according to our unpublished observations (31). Also, giving direct nicotine to the body parenterally leads to much greater nicotine intake for the body compared to administering the same dose of nicotine orally or via inhalation. When the studies reporting typical smokers who systemically absorb about 0.3 mg nicotine/kg BW per day based on the average daily nicotine consumption of 17 cigarettes (nearly one package) in the U.S.A, studies giving nicotine directly to the systemic circulation by injection is like giving a toxic dose of nicotine to an individual, as it corresponds to smoking more than 10 packages of cigarette per day, which is impossible in daily life (32, 33). Parenteral high doses of nicotine may affect sperm and ovarian follicle function but the low dose nicotine in our study probably has no adverse effect on fertility, since the daily low dose nicotine consumption did not affect delivery rates and number of pups at each delivery of the pregnant rats under nicotine exposure in comparison to controls.

Many guides that deal with NRT administration in pregnancy are contradictory. In the 2002 edition of Current Guidelines for Smoking Cessation in Pregnancy which deals with quitting smoking in pregnancy, it is reported that all nicotine gums and bands are contraindicated during pregnancy and it is stressed through various animal experiments that they may harm the fetus (34). On the other hand, the American Agency for Health Care Policy and Research supports the use of NRT since quitting nicotine abruptly during smoking cessation may lead to withdrawal symptoms and severe consequences in the fetus even though the efficiency of NRT in pregnant women is not known for certain (35).

In conclusion, contrary to common belief, the results of this study show that low dose nicotine does not cause the intrauterine growth retardation in the fetus frequently seen in smokers. Its chronic usage in the later period may influence growth and development negatively. Using maternal nicotine during pregnancy and lactation periods, even in small doses, may be effective in developing addiction in the new-born fetus. For this reason, nicotine replacement therapy for pregnant women intendinf to quit smoking, but can’t accomplish this with physiological and behavioral methods, may be recommended after taking into account the advantages and disadvantages of nicotine intake if they are not able to stop smoking. Therefore; pregnant women using NRT instead of smoking are protected from exposure to the other harmful materials in cigarette.

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Conflict of interest

No conflict of interest was declared by the authors.

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