

## The effects of exposing rats to cigarette smoke on milk production and growth of offspring

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### Abstract

**Objectives:** To analyze the effects of exposure to cigarette smoke during gestation and lactation on the milk production of rats and on the weight gain and linear growth of their offspring.

**Methods:** Three groups of female rats were studied during gestation and lactation: 15 rats were exposed to cigarette smoke and air flow, 18 rats were handled, i.e., exposed to compressed air flow, and 18 rats were used as controls. Newborn rats were measured and weighed every 5 days, from the first to the 15th day. Milk production was estimated by 1-hour milk extraction and weight gained by litters.

**Results:** The offspring of rats exposed to cigarette smoke weighed less and were shorter at birth. During lactation, the offspring of rats exposed to smoke and also of rats that had merely been handled gained less weight than the control group. Milk production gauged by the 1-hour extraction method was reduced in the group exposed to smoke and, to a lesser extent, also in the group that had been handled. Milk production estimated according to the pups' weight gain was reduced equally in the groups exposed to smoke and merely handled, when compared to the control group.

**Conclusions:** Exposure to cigarette smoke compromised the birth weight and birth length, but during lactation, handling also compromised weight gain of offspring. Handling and, to a greater extent, exposure to tobacco, were prejudicial to milk production.

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### Introduction

Smoking can affect several phases of human reproduction, including lactation.<sup>1</sup> The children of mothers who smoke gain weight at a slower velocity than the children of non-smokers, suggesting that smoking may affect milk production.<sup>2</sup> Studies of daily milk production between 1 and 3 months into lactation have shown that daily milk production is significantly lower in smoking mothers. When weight gain was measured over a 14-day period, it was observed that the children of smokers exhibited 40% lower mean weight gain than the children of non-smokers.<sup>3</sup>

Active maternal smoking habits have been linked with breastfeeding failure, reducing milk production and breastfeeding duration,<sup>4,5</sup> both exclusive and mixed,<sup>2</sup> and the reduction in breastfeeding duration is proportional to the intensity of maternal smoking.<sup>6-8</sup>

Although the harmful effects of active smoking have been accepted as impacting several phases of the female reproductive process, an ever growing number of studies are referring to similar effects caused by passive smoking. The relative risk (RR) of a mother exposed to passive smoking giving birth to a low birth weight child was 2,17 (95%CI

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1.05-4.50) when compared with mothers who had no contact with cigarettes.<sup>9</sup> It appears that fetuses,<sup>10</sup> newborn infants and young infants<sup>11</sup> are most at risk from exposure to passive smoking.

Although experimental studies have analyzed the effects of smoking on lactation,<sup>12</sup> their results have been divergent. Working from the hypothesis that smoking affects both postnatal growth and weight gain of offspring, the objective of this experimental study with rats was to analyze the effects of cigarette smoke on the dams' milk production and on their litters' weight gain and linear growth.

## Methods

This study employed an experimental rat model of exposure to tobacco smoke, analyzing several parameters,<sup>13</sup> including linear growth and weight gain of pups and dams' milk production.

The sample size calculation was made based on a 5% level of significance ( $p < 0.05$ ) and a beta error of 0.1. The formula used was  $N = [(z_a - z_b) \theta]^2 / \delta^2$ , where  $z_a$  and  $z_b$  are  $z$  scores from a normal curve associated with the alpha and beta values,  $\theta$  is the standard deviation and  $\delta$  the difference considered significant. The resultant sample size was a minimum of 15 animals per group.

The sample consisted of 51 Wistar strain virgin female rats aged 4 months (*Ratus norvegicus*), provided by the Biotério Central at the Universidade Federal de Mato Grosso and maintained at  $22 \pm 2$  °C temperature, 40 to 60% humidity and with a cycle of 12 hours darkness and 12 hours light. Animals were accepted at weights between 230 and 260 g and were fed on Nuvilab rations (Nuvital, Curitiba, PR) and water *ad libitum*.

The animals were separated into three groups:

- Group S: animals exposed to cigarette smoke in compressed air flow for 15 minutes, twice a day, during gestation and lactation ( $n = 15$ );
- Group A: animals handled and exposed to compressed air for 15 minutes twice a day, during gestation and lactation ( $n = 18$ );
- Group C (control): animals that were not handled, except for the minimum necessary for weighing ( $n = 18$ ).

The animals in group S were exposed to smoke from Marlboro® (Philip Morris) brand cigarettes. Each cigarette contained 0.8 mg of nicotine, 10 mg of tar and 10 mg of carbon monoxide (measured by sampling, Labstat Laboratory, Canada).

The exposure system employed and assessment of system functionality and of exposure markers – cotinine and carboxyhemoglobin – have been described previously.<sup>14,15</sup> The exposure system consisted of two wooden chambers

separated by a perforated wall. In the first chamber (combustion), the cigarettes were burnt, passively, and the animals were placed in the second chamber (inhalation). During exposure compressed air fed the combustion and directed the smoke flow into the inhalation chamber and from there to an exit.

The animals in groups S and A were subjected to inhalation for 15 minutes, twice a day, with a 12 hour interval, from confirmation of pregnancy, by vaginal smear, until the 17th day of lactation. During exposure, the animals in group S inhaled the smoke from cigarettes that were completely burnt and ventilated by compressed air flow (10 L/minute). Ten cigarettes were used per day (five cigarettes per exposure), i.e. two cigarettes/animal/day. The animals in group A were put in an exposure system that was reserved exclusively for that group, with a similar compressed air flow, at the same frequency and duration and at the same times of day as the animals in group S.

Litters were reduced to eight newborn rats, eliminating the excess at random. Reductions in litter size were tolerated to a minimum of seven pups. The newborn rats were numbered between 12 and 24 hours after birth, and once again on the 10th day of life. Weight and length (measured from nose to tip of tail with the pup extended on a flat surface) were obtained according to the protocol published by Silva et al.<sup>14</sup> Weights and lengths were measured on the first, fifth, 10th and 15th days of life, always at the same time of day, and weight was also recorded in the morning on the 12th day of life. Weight gained during lactation was measured according to weight accumulated or by the difference between birth weight and the weight measured on the days mentioned.

Milk production was estimated according to methods described by Morag<sup>16</sup> and Sampson & Jansen.<sup>17</sup> Using the Morag<sup>16</sup> method, the difference in the pups' weights before and after suckling was recorded (milk extraction measurement). On the 12th day of lactation, pups were separated from dams for 12 hours and, at the end of this period, were weighed (initial weight). Next, immediately after the dams had been exposed to smoke or air or not exposed (group C), the pups were returned to the dams, allowed to suckle for 1 hour and after weighed once more (final weight). Milk production, in g/pup, was defined as the mean weight gain in 1 hour for each litter.

The method published by Sampson & Jansen<sup>17</sup> was also used, by which the period from the fifth to the 12th day after birth was studied, applying the following equation: milk production (g/pup/day) =  $0.0332 + 0.667(\text{weight}) + 0.877(\text{weight gain})$ , where weight is the weight on the last day of the period and weight gain is the difference between the initial weight and the weight on the last day of the period.

Data were tabulated on Excel, and statistical analyses performed using SPSS version 9.0. The Lavene test was employed to verify the behavior of numerical variables. Parametric analyses were performed using ANOVA or Student's *t* test, as appropriate. The level of significance adopted was  $p < 0.05$ .

The research project was approved by the Ethics Commission responsible for research (CAPPesq) at the Hospital das Clínicas and Medical Faculty of the Universidade de São Paulo, hearing number 777/01 on 25th October 2001.

## Results

Table 1 contains the weights of the rat pups from birth up until the 15th day of life. It will be observed that the pups of dams in group S had lower birth weights than those of the control animals, and that this difference persisted throughout lactation. The pups of group A dams were born weighing more than those from group S, but this difference lost significance from the 10th day of lactation onwards. On the 15th day of lactation, the pups of group A animals have comparable weights to those of group S animals and weigh less than the control group's pups.

The pups of group S dams were shorter than those of group C at birth and throughout lactation (Table 2). The offspring of rats in group A were born longer than the

offspring of rats in group S, but this difference lost significance from the 10th day of lactation onwards. When the two groups that had not been exposed to cigarette smoke were compared (compressed air and control groups), no differences were observed between their pups' lengths on any of the data collection days.

Comparison of the weight gained by the rat pups over their first 15 days of life demonstrates that the control group gained more weight than group S throughout lactation. In contrast, the offspring of group A dams gained weight at a similar rate to the controls at the start of lactation, but this rate of gain reduced significantly as lactation progressed, which was detected on the 15th day of life and meant that, when compared with the pups of group S dams, group A pups were not significantly different in terms of weight gain during lactation: S = 16.91 g ( $\pm 1.91$ ); A = 17.26 g ( $\pm 2.09$ ); C = 18.81 g ( $\pm 2.26$ ) (S vs. C;  $p < 0.05$ ).

Milk production data (Figure 1), as assessed on the 12th day of lactation according to the procedure described by Morag,<sup>9</sup> demonstrated that the offspring of group S received less milk than the offspring of rats in the other two groups and that the offspring of group A rats received less milk than those of the controls (Figure 1, first graph).

The results of evaluation of milk production, assessed from the fifth to the 12th day of lactation using the method

**Table 1** - Mean weight (g) and standard deviation for the litters of rats exposed to cigarette smoke and compressed air compared with controls, at birth, and on the fifth, 10th and 15th days of life

	n	Birth	5th day	10th day	15th day
Group					
S	15	5.50 (0.28)	9.46 (0.76)	17.57 (1.19)	22.42 (1.86)
A	18	5.89 (0.47)	10.45 (1.06)	18.19 (1.51)	22.93 (1.87)
C	18	5.87 (0.36)	10.62 (1.16)	19.23 (1.99)	24.73 (2.45)
Test					
ANOVA		$p < 0.01$	$p < 0.01$	$p < 0.05$	$p < 0.01$
<i>t</i> test					
C x S		$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
C x A		NS	NS	NS	$p < 0.01$
S x A		$p < 0.01$	$p < 0.01$	NS	NS

A = compressed air; C = controls; NS = nonsignificant; S = cigarette smoke.

described by Sampson & Jansen,<sup>17</sup> demonstrate that rats exposed to cigarette smoke and rats exposed to compressed air did not exhibit any differences in milk production, but that both these groups produced less milk than the rats in the control group (Figure 1, second graph).

### Discussion

Assessment of the weight gain and linear growth of newborn rats demonstrated that the difference in weight between the offspring of rats exposed to cigarette smoke and controls remained even when birth weight was subtracted from total weight gain by means of analysis of accumulated weight gain. When accumulated weight gain during lactation was compared for the pups of groups exposed to cigarette smoke and the pups of those exposed to compressed air, no statistically significant differences were observed between means. These data suggest that, when attempts are made to cancel out the effect of handling (exposing animals to compressed air only), the weight loss observed at birth among the litters of mothers exposed to smoke disappears during lactation.

In this study, weight gain was assessed in the offspring of female rats exposed to tobacco throughout pregnancy and practically the entire lactation period, attempting to thereby mimic the condition of human smoking. Since these

reproductive periods were not studied in isolation, the effects observed during lactation may be a reflection of maternal and fetal alterations resulting from the exposure during gestation. It is known that nicotine interferes in the build up of lipids during pregnancy, and may reduce the mother's capacity to meet the energy demands of lactation, particularly when nutritional intake is restricted.<sup>18</sup> Similarly, maternal intake, analyzed both qualitatively and quantitatively, is of great importance during lactation, both for the mother's health and for successful lactation, in addition to being essential for the growth of her pups.<sup>19</sup>

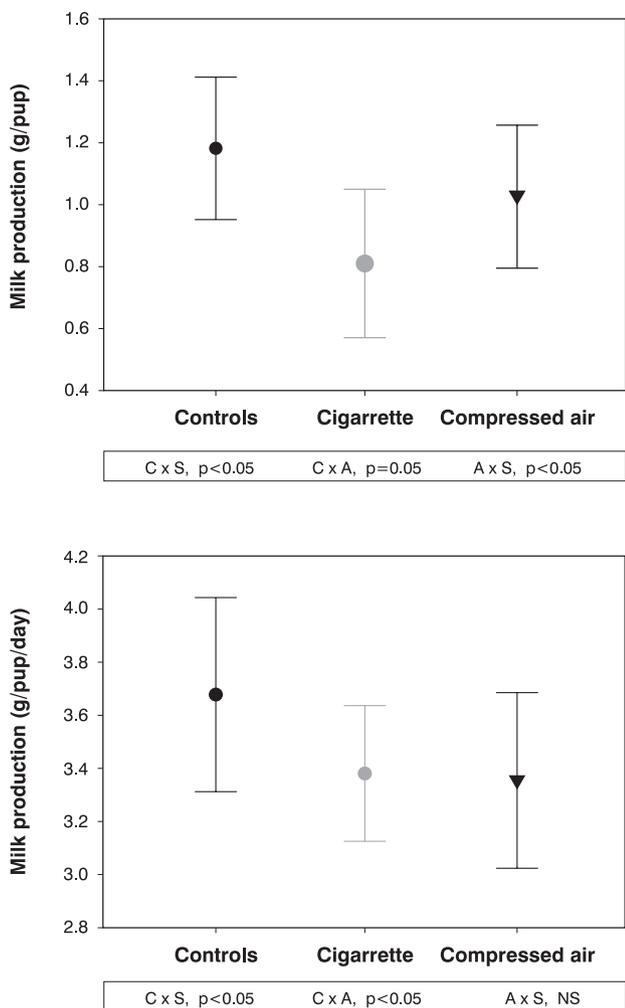
Newborn rats given nicotine injections during gestation and lactation exhibited reduced weight gain and linear growth at the end of the first half of lactation.<sup>20</sup> In contrast, newborn rats given nicotine during gestation, but not during lactation exhibited reduced birth weight, but with no significant difference in weight at weaning after 21 days.<sup>21</sup>

Studies with humans have shown that, although smoking during pregnancy is associated with newborn infants whose weights are lower when delivered, after birth, these children tend to catch up with the ideal growth curve.<sup>22</sup> It would appear that the effects of smoking are associated more with interuterine exposure than with postnatal exposure (passive smoking) during the early stages of infancy.<sup>23</sup>

**Table 2** - Mean values (cm) and standard deviations for the lengths of pups of rats exposed to cigarette smoke or compressed air compared with controls, at birth and on the fifth, 10th and 15th days of life

	n	Birth	5th day	10th day	15th day
Group					
S	15	6.78 (0.28)	8.80 (0.76)	11.60 (1.19)	13.96 (1.86)
A	18	6.94 (0.18)	9.26 (0.38)	11.83 (0.38)	14.20 (0.51)
C	18	6.92 (0.11)	9.20 (0.32)	11.94 (0.45)	14.49 (0.61)
Test					
ANOVA		p < 0.05	p < 0.05	NS	p < 0.05
t test					
C x S		p < 0.01	p < 0.05	p < 0.05	p < 0.05
C x A		NS	NS	NS	NS
S x A		p < 0.05	p < 0.05	NS	NS

A = compressed air; C = controls; NS = nonsignificant; S = cigarette smoke.



A = compressed air; C = controls; NS = nonsignificant; S = cigarette smoke.

**Figure 1** - Mean values and standard deviations for milk production of rats exposed to cigarette smoke and compressed air, compared with controls: in g/pup according to the 1-hour milk extraction method (first graph) and in g/pup/day (Sampson & Jansen, 1984) measured from the fifth to the 12th day of lactation (second graph)

The data analyzed here show that the animals exposed to compressed air were born with similar weights to the controls. However, during lactation they gained less weight than the controls, and a similar amount to the pups of the group exposed to cigarette smoke. The hypothesis therefore arises that the lower corrected weight gain in the two groups of exposed animals compared with the unhandled animals (controls) may not be the result of gestational events, but of factors that occurred during lactation, such as stress.

The mean values of milk extraction recorded for the control group were 1.18 g/pup, greater than figures reported by Morag,<sup>16</sup> which were 0.9 g/pup, but smaller than those described by Lau,<sup>24</sup> which were 1.35 g/pup. These figures, 15% greater, may be the result of different animal lineage, diet and accommodation. Comparing the results of the three

study groups, it will be observed that the control group extracted the greatest volume of milk, followed by the compressed air group and then by the group exposed to cigarette smoke. According to this method, although milk production was lower in the groups exposed to cigarette smoke and compressed air than in the control group, production was greater in the group exposed exclusively to compressed air in comparison with the group exposed to cigarette smoke.

Analyzing milk production according to the method proposed by Sampson & Jansen,<sup>17</sup> it was observed that the dams in groups S and A produced less milk than those in the control group and did not exhibit differences when compared with each other. Grigor<sup>25</sup> estimated milk production indirectly from the sum weight gain of animals over 24 hours, with insensible losses accounted for in the form of the weight lost by animals fasting for an equal period. Their results produced a figure for milk production of 46 g a day in litters reduced to 10 individuals. Grigor<sup>25</sup> also applied the equation described by Sampson & Jansen<sup>17</sup> and found a milk production estimate that was 10% lower. These values are similar to those found in the present study, which were arrived at using the same equation, although corrected for litters reduced to eight members.

It is known that the method proposed by Sampson & Jansen<sup>17</sup> is an indirect measure of milk production, estimated from the mean daily weight gain of the litter. The calculation takes into consideration the interference provoked by insensible losses (perspiration and excretions) and assumes constant weight gain from the fifth to the 15th days of life.

While the weight gain of a standardized litter can be used as a qualitative index of milk production, it cannot be used to accurately measure milk production, since the pups' maintenance requirements are not taken into account, and neither are possible variations in maternal nutrient intake that may alter the milk's composition.<sup>19</sup>

The comparison of results from two different measurements of milk production demonstrated that, although the measurement taking throughout lactation did not detect a difference between the groups exposed to cigarette smoke and to compressed air, the measurement calculated according to the Morag<sup>16</sup> method demonstrated an independent effect from the cigarette smoke. Since this measurement reflects milk production during the previous 12 hours, the milk produced during the period that mothers were separated from their litters may have been influenced by the two exposures that took place during this interval. Nevertheless, the long term measurement did not reveal any difference between the S and A groups, which suggests that the animals exposed to tobacco maintained milk production during the interval of exposure to cigarette smoke. This

finding is, in part, in agreement with the results of a hormonal assessment, which showed that the animals exposed to cigarette smoke did not demonstrate any differences in prolactin secretion.<sup>13</sup> Therefore, the lower amount of weight gained by the litters in the smoking group in 1 hour of suction may be caused by reduced ejection of milk by mothers.

It is known that milk ejection can be affected by stimuli that cause stress. Liberation of milk has been observed to reduce when female rats are subjected to visual, olfactory and auditory stimuli or are restricted physically.<sup>26</sup> The mechanisms involved are thought to be the result of activation of the peripheral sympathetic adrenal system and of a reduction in the number of oxytocin pulses. Sympathetic activation causes vasoconstriction of mammary glands making it harder for oxytocin to access the myoepithelial cells, provoking an increase in the tonus of mammary ducts and influencing the dynamics of intramammary pressures.<sup>27,28</sup> Furthermore, part of this process of sympathetic stimulation is a cerebral component that inhibits the oxytocin stimulus responsible for ejection of milk.<sup>29</sup>

However, the effects of stress on lactation are complex. Depending on the duration of stress and on its site(s) of action, suppressed lactation may be the result of a reduction in synthesis or ejection of the milk. The duration of the effects observed may vary, depending on whether the stress is acute or prolonged, affecting the action of prolactin – synthesis – or the action of oxytocin – ejection of milk.<sup>30</sup>

In contrast with the results observed during lactation, the two groups of animals that were not exposed to cigarette smoke did not differ in terms of birth weight, denoting an effect from tobacco exclusive to gestation. Overall, the weight gain and milk production data suggest that the effect on lactation of stressing the animals during experimental handling was similar to the method of tobacco exposure adopted, and that the observed effect of the cigarettes on milk extraction may be overcome by litters in the intervals between exposures. For the model adopted, lactation was sensitive to animal stress, in contrast with gestation, which was only sensitive to the effects of exposure to cigarettes.

In this study, exposure to cigarette smoke reduced weight and length at birth. This effect was independent and attributable to tobacco. During lactation the female rats exposed to tobacco smoke produced smaller volumes of milk, their pups captured less milk and exhibited slower weight gain and less postnatal linear growth in relation to the control group. During lactation, handling and exposure to compressed air prejudiced the weight gain of offspring. Milk extraction was reduced by handling and, to a greater extent, by exposure to cigarette smoke, but, over the lactation period, the effect of cigarette smoke in reducing milk production was no different from that of handling.

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