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Original Article

The impact of tobacco smoking on physical activity and metabolism in mice

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Abstract: Cigarette smoking can increase the risk of many respiratory and chronic systemic diseases. Particularly, cigarette smoke produces toxic particulate matter (PM), which is harmful to the smokers. Although previous studies have demonstrated the toxicity of cigarette smoke PM and its relationship with disease pathogenesis, systematic data for the impact of cigarette smoke PM on physical activity and metabolism in animals are still lacking. In this report, the C57/B6 mice were exposed to cigarette smoke PM in a smoking chamber coupled with the analysis of metabolic changes and physical activity in metabolic cages at indicated time, for a period up to 12-month-old of age. The mice became excited following short period (e.g., 3 months) but listless after long-term cigarette smoke PM exposures (e.g., 9 or 10 months), as manifested by the changes of drink/food intake and daily activities along with increased oxygen consumption and CO_2 accumulation. Our data suggest that particulate matter originated from cigarette smoke impairs metabolism and physical activities.

Keywords: Cigarette smoking, tobacco, physical activity, metabolism, particulate matter

Introduction

Tobacco smoking is harmful to human health and is a worldwide problem. The World Health Organization (WHO) estimates that tobacco use kills more than 7-million people each year. More than 6-million of those deaths are the result of direct tobacco use, while approximately 890,000 of the deaths are due to the exposure of second-hand smoke in non-smokers [1]. Particularly in China, smoking is not completely prohibited in some public places, which renders a large proportion of the population passively exposed by second-hand or third-hand smoke involuntarily. Among which, women and children are unfortunately the main victims. Cigarette smoking harms nearly every organ of the body, causes many diseases, and damages the health of smokers in general [2, 3]. The main organ directly affected by cigarette smoking is the lung, and chronic obstructive pulmonary disease (COPD), asthma, as well as lung cancer are the most commonly reported respiratory diseases relevant to cigarette smoking [4].

Tobacco smoking has been suggested to affect many physical processes in humans [5, 6]. For example, smoking could cause laziness and depression along with decreased physical activity and nutritional intake [7], and it has also been reported to change the circulating metabolites relevant to disease development [8]. It is noteworthy that most of these data were collected from humans, and the related animal data such as in mice are lacking. Given the fact that collection of those related data in mice would be of highly important to fully address the impact of tobacco smoke on human health, we thus in the present report, conducted studies in C57/B6 mice to monitor the effect

of cigarette smoking on physical and metabolic activities.

Materials and methods

Animals and administration of tobacco smoke

The C57/B6 (B6, 6 wk-old) mice purchased from the HFK Bioscience (Beijing, China) were used in the study. All mice were acclimatized for 2 weeks and fed under normal chow, and then randomly grouped into no-smoking and smoking group with each group containing 25 mice, respectively. Tobacco administration was carried out in an automatic smoking system (HRH-SM120, Beijing, China) equipped with a chamber containing steady concentration of tobacco smoke particulate matter (PM). In general, mice were put into the chamber with 1,000 mg/m^3 concentration of tobacco smoke PM for a 2 h period of time each day, 5 days per week, and lasted up to 12-month-old of age. Four mice from each group were randomly selected for metabolic analysis as described below at the 3rd, 4th, 9th, and 10th month of tobacco smoke PM administration.

Metabolic studies

For the analysis of metabolic index, the mice were placed in metabolic cages individually connected to a comprehensive laboratory animal-monitoring system (Columbus Instruments, Columbus, OH) [9]. The mice were acclimatized to respiratory chambers for 48 h, followed by real-time recording of the data for water intake, food intake, oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory exchange ratio (RER, the ratio of VCO_2/VO_2), total physical activities (movements or running) in X-, Y- and Z-axis [10].

Tobacco

The tobacco employed in the study was provided by the China Tobacco Jiangxi Industrial Co., Ltd. (Nanchang, China). The tobacco was contained in cigarettes without additive agents. Each cigarette contained 50 mg of nicotine, which is equivalent to 30 g of tobacco leaf [11]. Under normal conditions, one cigarette burned continuously for 20 minutes with steady smoking.

Statistical analysis

All data were expressed as mean ± SEM. Prism 5.0 software (GraphPad, La Jolla, CA) was used for statistical analysis of all data using the Student *t* test where appropriate. In all cases, $P<0.05$ was considered with statistical significance.

Results

The impact of cigarette smoking on water and food intake

We first monitored the changes of water and food intake after sustained exposure of cigarette smoke PM by randomly analyzing 8 mice from each group at each time point. Surprisingly, we failed to observe a significant change in terms of water and food intake between two groups of mice after 3 (**Figure 1A**, **1B**), 4 (**Figure 1C**, **1D**), 9 (**Figure 1E**, **1F**) and 10 months (**Figure 1G**, **1H**) of cigarette smoke PM exposures, although the mice after 3 months and 9 months of cigarette smoking manifested a trend of higher water and food intake, and a trend of lower water and food intake in mice after 4 months of cigarette smoking.

Metabolic changes in mice after administration of tobacco smoke PM

Next, we sought to monitor the changes of metabolic activities. Interestingly, the mice after 3 (**Figure 2A**, **2B**) and 4 months (**Figure 2C**, **2D**) of exposures of cigarette smoke PM did not show a significant change in terms of oxygen consumption and CO_2 accumulation compared to mice in the control group. Remarkably, mice with sustained exposures of cigarette smoke PM up to 9 (**Figure 2E**, **2F**) and 10 months (**Figure 2G**, **2H**) showed significantly higher oxygen consumption and CO_2 accumulation than that of mice in the control group during the night time. Given the fact that the respiratory exchange ratio (RER) serves as a metabolic index to comprehensively reveal the activities of oxygenic metabolites, we thus next compared the differences of RER between two groups of mice. Unexpectedly, we failed to detect a significant difference of RER between two groups of mice after 3 (**Figure 2I**), 4 (**Figure 2J**), 9 (**Figure 2K**) and 10 months (**Figure 2L**) of cigarette smoke PM exposures. Combining the RER levels with the O_2 consump-

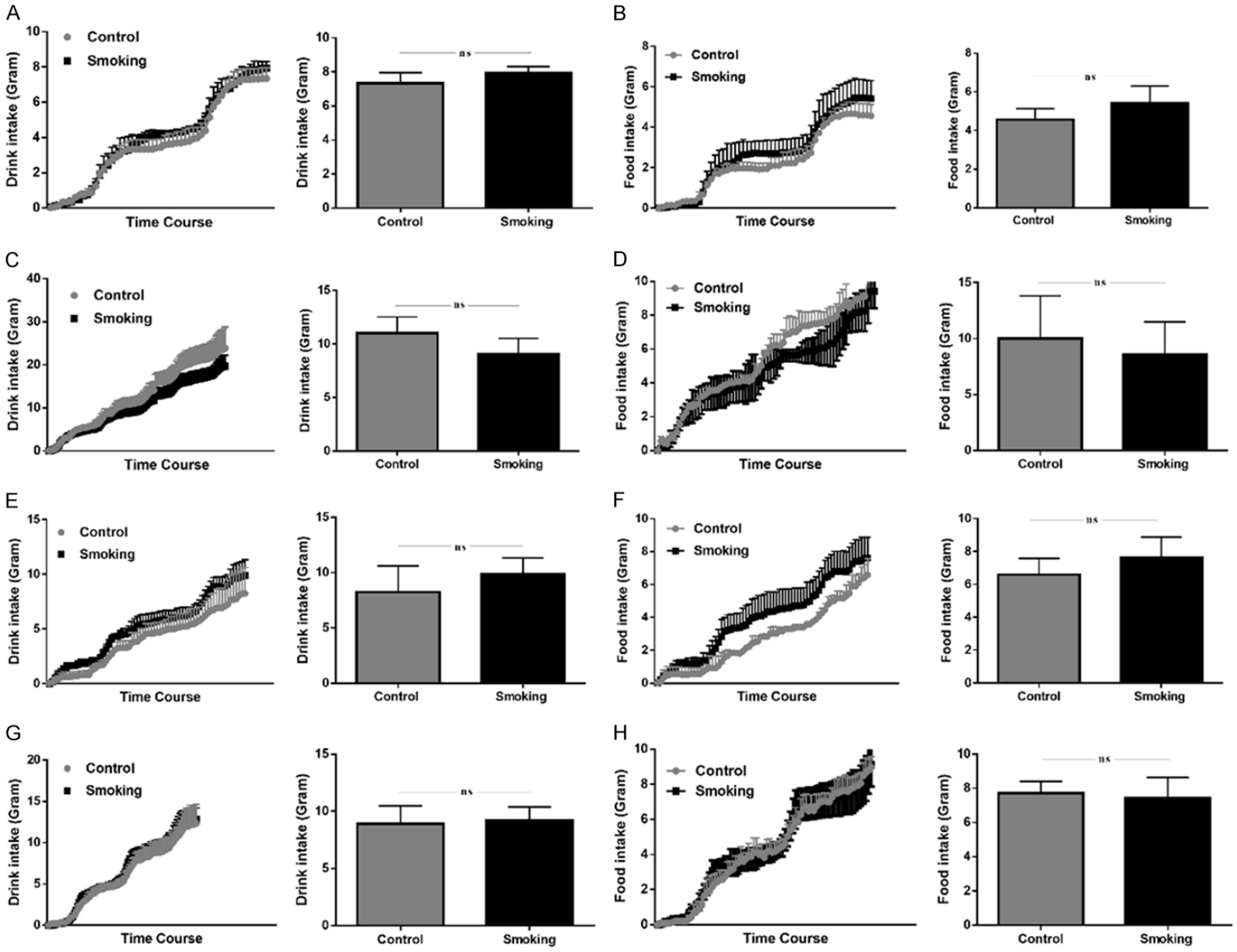
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Figure 1. The impact of cigarette smoking on water and food intake. (A and B) Results for water (A) and food (B) intake following 3 months of cigarette smoke PM exposures. (C and D) Results for water (C) and food (D) intake following 4 months of cigarette smoke PM exposures. (E and F) Results for water (E) and food (F) intake following 9 months of cigarette smoke PM exposures. (G and H) Results for water (G) and food (H) intake following 10 months of cigarette smoke PM exposures. Left panels show the results for real-time monitoring of water and food intake, whereas right panels present mean value of water and food intake. Four mice were randomly selected from each group at each time point for the study.

tion and CO_2 accumulation data, our results suggest that long-term smoking exposure may attenuate anaerobic metabolites.

The effect of cigarette smoking on physical activities

The above data prompted us to examine the movement of those mice, which implies the strength of physical activities. To this end, we recorded their movement in the X, Y and Z axes. Interestingly, at the early stage (i.e., following 3-month of cigarette smoke PM exposures), the mice were more active than the control mice as evidenced by the significant increase of movement in the X (**Figure 3A**), Y (**Figure 3B**) and Z axes (**Figure 3C**) during the night time. However, as the cigarette smoke PM exposure time progressed, the mice became less active than the controls. Specifically, after 4-month of cigarette smoke PM exposures, the mice were less active at the Y (**Figure 3E**) and Z axes (**Figure 3F**), but did not show a significant change for their movement in the X axe (**Figure 3D**). However, following 9-month of sustained cigarette smoke PM exposures, the mice became more active in the Y axe (**Figure 3H**) both in the day and night time, but no significant change was observed in the X (**Figure 3G**) and Z axes (**Figure 3I**). In sharp contrast, after 10-month of cigarette smoke PM exposures, all mice displayed reduced movement in all axes (**Figure 3J-L**) during night time. Taken together, these results suggest that short-term smoking renders the mice more active, while the mice become indolent after long-term of cigarette smoke PM exposures.

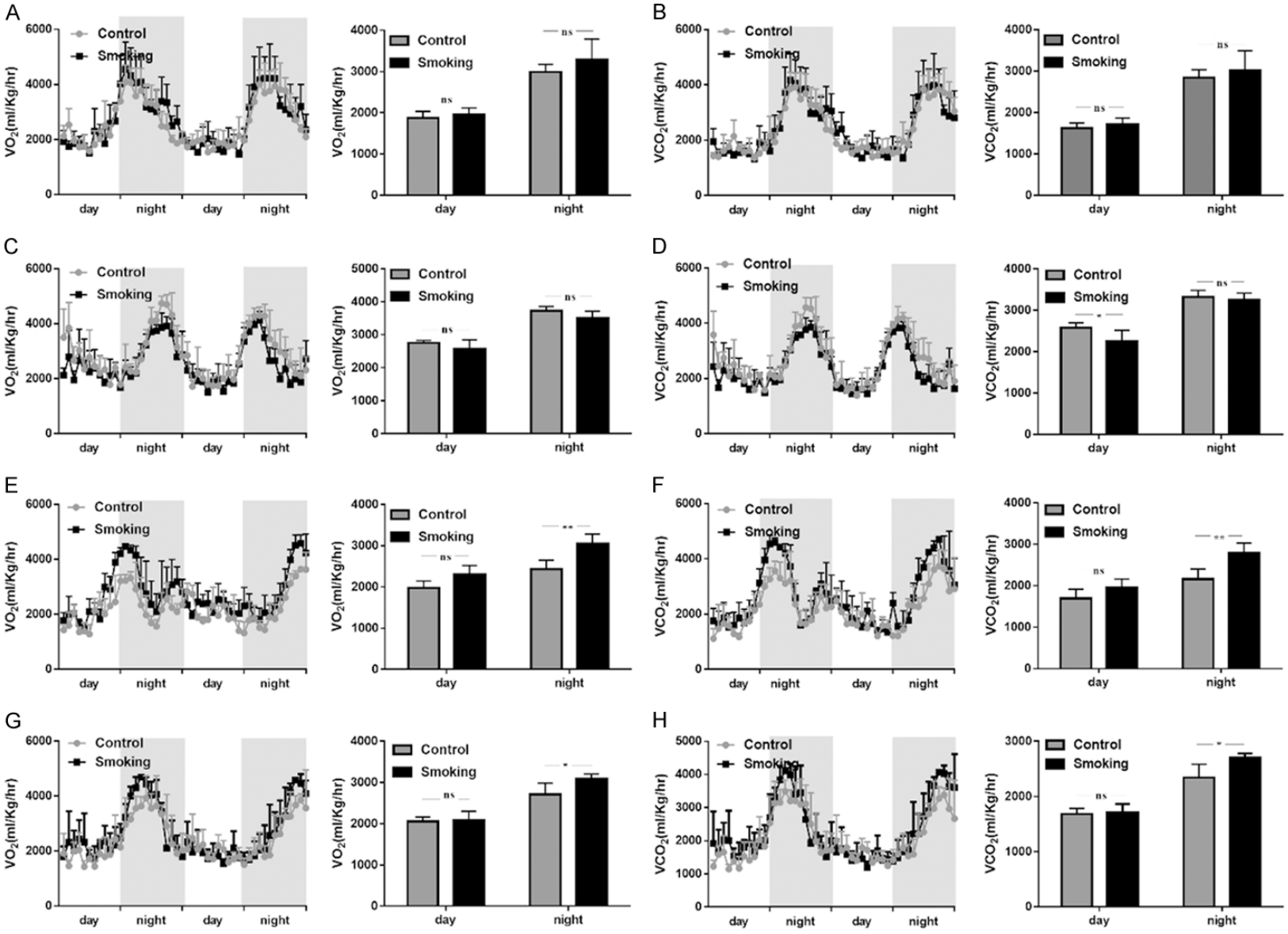
The change of body weight following administration of cigarette smoke PM

Finally, we recorded the weight changes following cigarette smoke PM exposures every four weeks and lasted for 40 weeks. During the first 4-week of cigarette smoke PM exposures, no significant difference was observed in terms of body weight between two groups of mice. However, the cigarette smoke PM exposed mice displayed a significantly lower body weight than that of control mice after 4-week of administration. Particularly, unlike control mice, after 12-week of sustained cigarette smoke exposures, a steady decrease of body weight was observed in all experimental mice (**Figure 4**). Of note, 5 mice died following 10 months of cigarette smoke exposures. Two died from lung cancer, while the rest were died from emphysema and dyspnea according to the pathological analyses (data not shown).

Discussion

In the present report, we monitored the impact of sustained cigarette smoke PM exposures on metabolic activity in mice for a period of ten months. Metabolic markers including the consumption of food, water and O_2, and the accumulation of CO_2, as well as physical activities, were recorded continuously under both day and night time. It was interestingly found that cigarette smoke PM exposures did not result in a significant change in terms of water and food intake, O_2 consumption, and CO_2 accumulation. In sharp contrast, mice with short term of cigarette smoke PM exposures manifested increased physical activity, while long term of exposures led to deceased physical activities as featured by a significant reduction of movement in the X, Y and Z axes coupled with body weight reduction.

Generally, food and water consumption are direct metabolic markers, and body weight is directly associated with metabolic activities. Our studies provided evidence indicating that mice with sustained cigarette smoke PM exposures manifested an N curve for body weight, which was always less than that of control mice, although no significant difference was observed between two groups of mice during the first 4-week of cigarette smoke PM exposures. Given the fact that the cigarette smoke PM exposed mice did not show a significant increase in terms of food and water intake, our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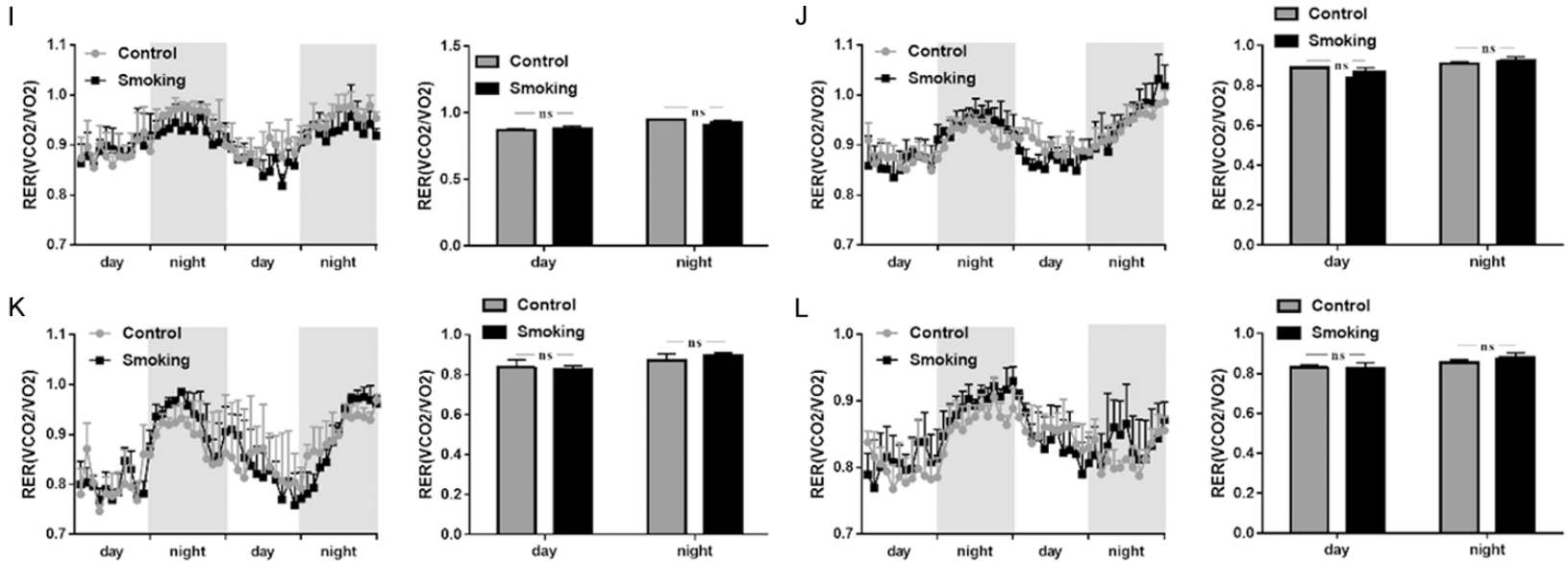
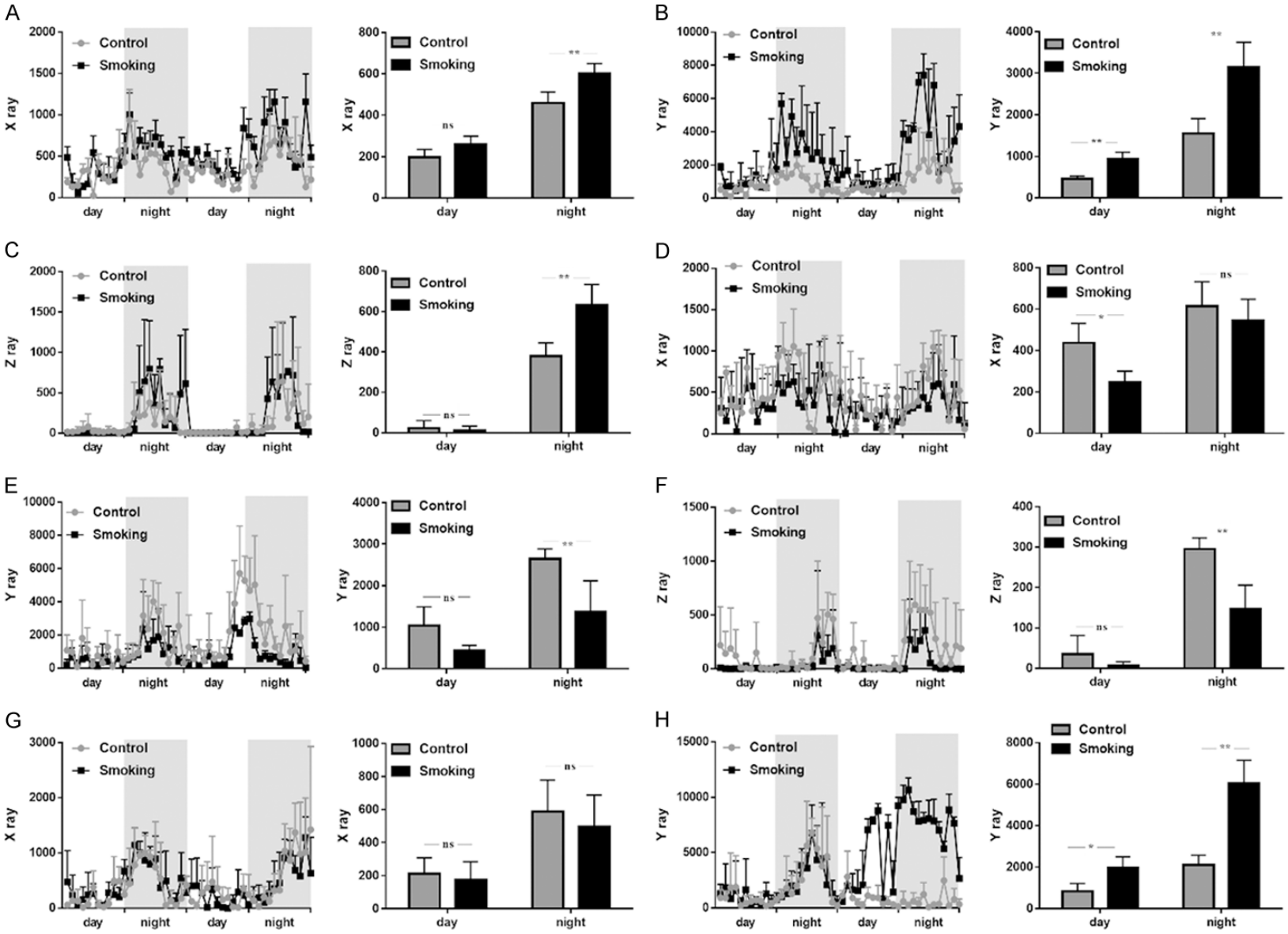


Figure 2. Metabolic changes in mice following sustained cigarette smoke PM exposures. (A and B) O_2 consumption (A) and CO_2 production (B) following 3 months of cigarette smoke PM exposures. (C and D) O_2 consumption (C) and CO_2 production (D) following 4 months of cigarette smoke PM exposures. (E and F) O_2 consumption (E) and CO_2 production (F) following 9 months of cigarette smoke PM exposures. (G and H) O_2 consumption (G) and CO_2 production (H) following 10 months of cigarette smoke PM exposures. Left panels show the results for real-time monitoring of O_2 consumption and CO_2 production, whereas right panels present mean value of O_2 consumption and CO_2 production. (I-L) RER levels following 3 (I), 4 (J), 9 (K) and 10 (L) months of cigarette smoke PM exposures. Left panels show the results for real-time monitoring of RER, whereas right panels present mean value of RER. Four mice were randomly selected from each group at each time point for the study. *, $P<0.05$; **, $P<0.01$.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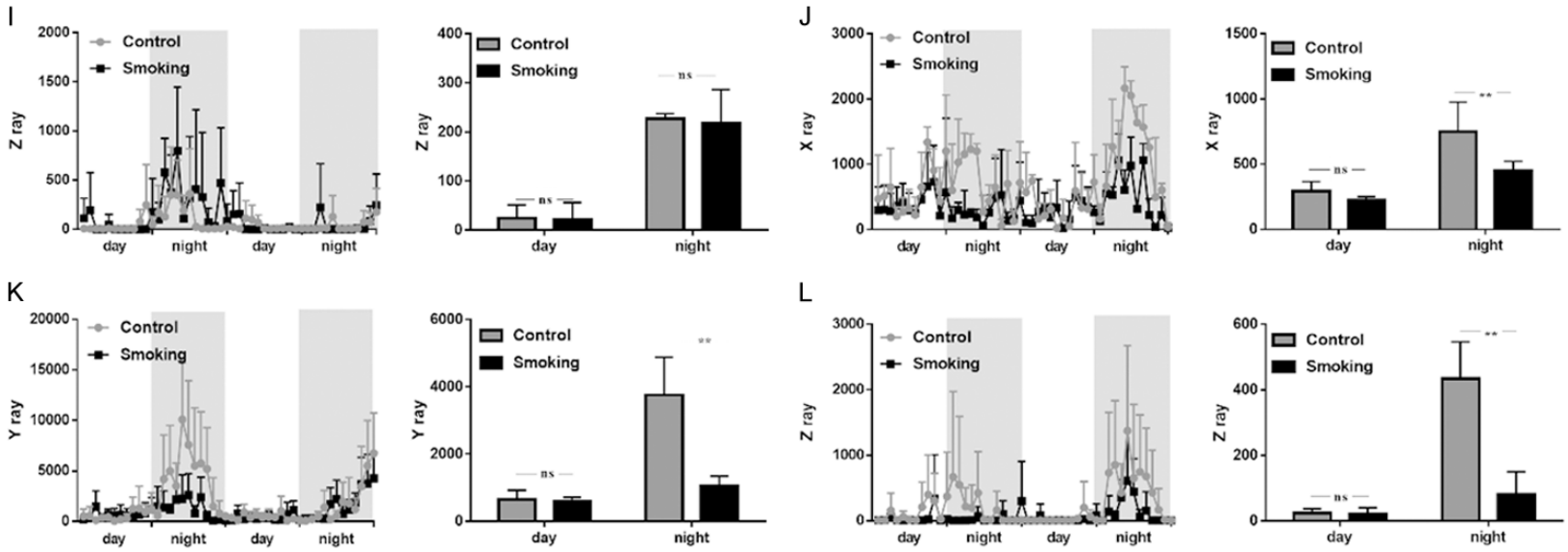

Figure 3. Results for measurement of physical activities in mice following sustained cigarette smoke PM exposures. (A-C) Results for measurement of movement in mice in X- (A), Y- (B) and Z-axis (C) following 3 months of cigarette smoke PM exposures. (D-F) Results measurement of movement in mice in X- (D), Y- (E) and Z-axis (F) following 4 months of cigarette smoke PM exposures. (G-I) Results measurement of movement in mice in X- (G), Y- (H) and Z-axis (I) following 9 months of cigarette smoke PM exposures. (J-L) Results measurement of movement in mice in X- (J), Y- (K) and Z-axis (L) following 10 months of cigarette smoke PM exposures. Left panels show the results for real-time monitoring of movement of mice in X-, Y- and Z-axis respectively, whereas right panels present mean value of movement of mice in X-, Y- and Z-axis respectively. Four mice were randomly selected from each group at each time point for the study. *, $P<0.05$; **, $P<0.01$.

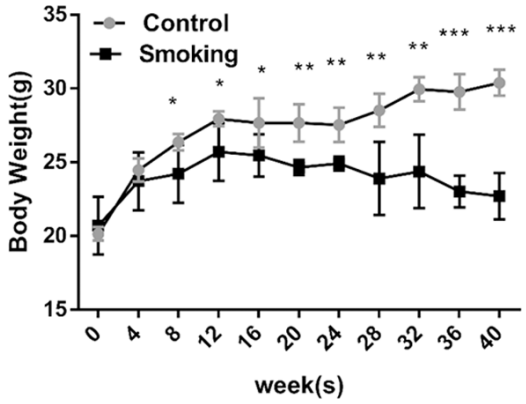

Figure 4. Comparison of temporal body weight changes between cigarette smoke PM exposed mice and control mice for a period of 40 weeks. Twenty-five mice were included in each group for the study. *, $P<0.05$; **, $P<0.01$; and ***, $P<0.001$.

data suggest that these mice consumed their stored energy to fulfill their physical activities, which is, somehow, inconsistent with the observations in the smoking populations [12-14]. Specifically, Jo and colleagues reported that smoking is associated with decreased food intake and lower body weight [15-18]. Cross-sectional studies also reported that smokers tended to eat slightly more than non-smokers or ex-smokers [19]. The mechanism underlying this phenomenon is unclear. One possible explanation is that nicotine exposure from cigarette smoke can change brain feeding regulation to reduce appetite *via* both energy homeostatic and reward mechanisms, by which it causes a negative energy state characterized by reduced energy intake and increased energy expenditure linked to a lower body weight [20].

To measure O_2 consumption and CO_2 accumulation, we assessed the aerobic respiration ratio in energy circulation. Under physiological conditions, the energy is generally provided by the aerobic metabolite pathway, which is the most efficient way to produce ATP in living cells. Therefore, the CO_2 level can reflect the total energy consumption of the body. In consideration of these two indexes, the RER levels reflect the metabolic state. Unexpectedly, we failed to observe a significant difference between two groups of mice in terms of the RER levels, but the O_2 consumption and CO_2 accumulation were significantly different. The increased O_2 consumption along with higher CO_2 accumulation in cigarette smoke PM exposed mice reflected an increased aerobic but decreased anaerobic respiration to fulfilled their energy needs [21], which could be a consequence of the respiratory excitation effect resulted from cigarette smoke PM exposures. Therefore, the mice could consume more energy to breathe, leading to an inefficient energy supply condition, and through which the metabolism changes into a negative direction, although the RER level remained balanced.

To assess the impact of cigarette smoke PM exposures on the intensity of physical activity, we monitored the distance of movement of the mice in the available space as previously reported [9, 10]. The X, Y, and Z axes were set to measure the movement forward and backward, left and right, and upward. As expected, our data indicate that sustained long-term smoking significantly affected the physical activity of mice, which was consistent with the observations in humans [22, 23].

In conclusion, we analyzed the metabolic changes in mice following sustained cigarette smoke PM exposures. Our data revealed that short-term cigarette smoke exposures could activate the metabolites, while long-term exposures exerted an inverse effect. Furthermore, cigarette smoking manifested a negative effect on the metabolites although the RER level remained balanced. Collectively, our study supports the notion that cigarette smoking impacts metabolism as evidenced by the altered O_2 consumption and CO_2 overproduction along with impaired physical activities.

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Disclosure of conflict of interest

None.

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