Effect of nicotine on body composition in mice

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Abstract

Nicotine induces weight loss in both humans and rodents consuming a regular diet; however, the effect of nicotine on body weight and fat composition in rodents consuming a high-fat diet (HFD) has not been well studied. Thus, this study examined the effect of nicotine vs saline on body weight and fat composition in mice fed with either an HFD (62% of kcal from fat) or a standard normal chow diet (NCD) for 7 weeks. Nicotine dose dependently reduced body weight gain in mice that consumed both diets, but this effect was significantly greater in mice on the HFD. Caloric intake was decreased in nicotine-treated mice. Estimates of energy intake suggested that decreased caloric intake accounted for all the reduced

weight gain in mice on an NCD and 66% of the reduced weight gain on an HFD. Computed tomography analysis for fat distribution demonstrated that nicotine was effective in reducing abdominal fat in mice that consumed the HFD, with nicotine treatment leading to lower visceral fat. The effect of nicotine on weight loss in mice on an HFD was completely blocked by mecamylamine, a nonselective nicotinic acetylcholine receptor (nAChR) antagonist, but only partially blocked by the $\alpha 4\beta 2$ nAChR partial agonist/antagonist, varenicline. We conclude that nicotine is effective in preventing HFD-induced weight gain and abdominal fat accumulation.

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Introduction

In a recent assessment of the mortality attributable to modifiable risk factors in the US adult population, it was found that tobacco use was responsible for 467 000 deaths (approximately one out of five) in 2005 (Fiore *et al.* 1999). Reduction in the rate of smoking could decrease over \$50 billion in health care costs within the United States (McGinnis & Foege 1999). Smoking is a major risk factor for devastating cardiovascular events such as myocardial infarction, sudden death, stroke, and peripheral vascular disease (Robbins *et al.* 1994, Murray & Lopez 1997, Wilson 1997), with a positive correlation between morbidity and mortality related to cardiovascular diseases and the number of cigarettes smoked per day (Kannel 1981). Importantly, the health risk associated with smoking is exaggerated by obesity, and smoking and obesity are the leading causes of morbidity and mortality worldwide (Haslam & James 2005, Chiolerio *et al.* 2008). The life expectancy of an obese smoker was 13 years less than that of a normal-weight nonsmoker (Chiolerio *et al.* 2008). Furthermore, smoking lowers body weight (BW) and BMI, which makes many people reluctant to quit

(Chiolerio *et al.* 2008). The finding that smoking lowers body weight but increases rates of diabetes, insulin resistance, and cardiovascular disease is an intriguing paradox that our laboratory is studying using both human and rodent models.

Smokers weigh less than nonsmokers of the same age and sex (Albanes *et al.* 1987), while cessation of smoking is usually accompanied by weight gain (Grunberg 1986, Klesges *et al.* 1989, Williamson *et al.* 1991). A comparison of 1911 homozygotic male twins, where only one of the siblings smoked, demonstrated that smokers weighed 2.5–5.0 kg less than nonsmokers (Eisen *et al.* 1993). Cigarette smoking is perceived as an effective strategy for weight control for many smokers. Approximately 75% of women and 35% of men reported that they would not tolerate a weight gain of more than 2.5 kg if they quit smoking (Pomerleau & Kurth 1996). One explanation for the reduction in body weight with tobacco smoking may result from decreased caloric intake and/or increased energy expenditure (Wager-Srdar *et al.* 1984, Grunberg 1986).

Although numerous rodent studies have provided convincing evidence for the inverse association between smoking/nicotine and body weight, the mechanisms underlying this

relationship are not well understood. Furthermore, smoking and nicotine intake are not equivalent; a number of studies suggest that nicotine has beneficial effects on body weight control, food intake, energy expenditure, as well as on improving cognition and preventing inflammation, whereas cigarette smoking is associated with increased risk factors for cardiovascular diseases and other morbidity and mortalities (Kannel 1981, Filozof *et al.* 2004, Wang *et al.* 2011). The effect of cigarettes can not only be solely explained by nicotine but also by many other components in the cigarettes, as well as by unhealthy lifestyles often exhibited by smokers (Grunberg 1985, Filozof *et al.* 2004). Some human and animal studies suggest that nicotine suppresses food intake (Bowen *et al.* 1986, Frankish *et al.* 1995), while other studies show that nicotine increases metabolic rate (Schechter & Cook 1976, Wager-Srdar *et al.* 1984, Hofstetter *et al.* 1986, Sztalryd *et al.* 1996). Winders & Grunberg (1990) showed that nicotine decreased weight gain and carcass fat, but did not affect food intake in rats.

We are concerned that some smokers know that smoking leads to lower weight and may erroneously conclude that they can eat whatever they want while smoking. Given that obesity is epidemic and that smoking/nicotine use is on the rise in many developing countries, it is clear that the co-occurrence of the smoking and weight gain has devastating effects on overall health throughout the world (Chiolero *et al.* 2008, Flegal *et al.* 2010). Additionally, there are two types of abdominal fat, visceral and subcutaneous, with the former being more metabolically active and associated with cardiovascular risk factors (Fox *et al.* 2007). Smokers have a lower amount of visceral fat than nonsmokers (Matsushita *et al.* 2010), although the effect of nicotine on fat distribution (visceral vs subcutaneous) in an animal model has not been determined.

To address these issues, in this study, we treated mice with different doses of nicotine or saline while they were on either a normal chow diet (NCD) or a high-fat diet (HFD) and examined changes in body weight, caloric intake, as well as measured fat distribution by computed tomography (CT) analysis and dual-energy X-ray absorption (DXA) scan analysis. Additionally, to determine the potential mechanism of nicotine action on alterations in body weight, we examined the ability of a nonselective nicotinic acetylcholine receptor (nAChR) antagonist, mecamylamine, and a partial nAChR agonist/antagonist, varenicline, to reverse the effects of nicotine on weight loss.

Materials and Methods

Animals and treatments

Male C57BL/6 mice weighing 22–24 g obtained from Taconic farm (Germantown, NY, USA) were used for all experiments. Mice were housed four per cage until the beginning of the experiments at which time they were individually housed with the exception that animals were

housed two per cage during the nicotine antagonists/partial agonist experiment. All mice had free access to water and food. All experiments using animals were performed in compliance with the NIH Guidelines for the Use of Animals in Research and approved by the Institutional Animal Care and Use Committee at Charles Drew University School of Medicine & Sciences (Los Angeles, CA, USA).

Mice were fed either an NCD with 5% fat (2.03 kcal/g; laboratory rodent diet #5001, LabDiet, Richmond, IN, USA) or an HFD with 60% of calories derived from fat consisting of 26.2% protein, 26.3% carbohydrate, and 34.9% fat (5.24 kcal/g; D12492, Research Diets, New Brunswick, NJ, USA) for 7 weeks. Mice on either diet received twice daily i.p. injections of either low-nicotine dose (0.5 mg/kg per BW per day, given twice daily) or high-nicotine dose (1.5 mg/kg per day, given twice daily), an escalating dose of nicotine (starting at 0.25 mg/kg per day, given twice daily and going up to 4.5 mg/kg per day by an increment of 0.5 mg/kg per day every 3 days), or saline. The escalating dose was designed to compensate for the development of nicotine tolerance. The 1.5 mg/kg per day dose is approximately equal to the amount of nicotine that a smoker smoking 50 cigarettes a day (2.5 packs) might be exposed to over the whole day (Armitage *et al.* 1968). For experiments using nAChR antagonists/partial agonists, mice were given twice daily i.p. injections of mecamylamine (2 mg/kg per day) or varenicline (2 mg/kg per day) concurrently with the nicotine injections and were only exposed to the HFD. In this experiment, mice were placed on the HFD 11 days before starting the injections.

Animals were weighed daily and the weight change from baseline was calculated. The amount of food consumed per mouse was measured daily. The cumulative caloric intake was calculated based on the kcal/g of each diet. For the antagonist study, mice were weighed and food consumption was determined every 3 days.

Drugs

(–)Nicotine liquid, mecamylamine hydrochloride, varenicline tartrate, and ketamine hydrochloride/xylazine hydrochloride solution were purchased from Sigma/Aldrich. Isoflurane was purchased from Attane (Bethlehem, PA, USA). Nicotine, mecamylamine, and varenicline were all diluted in saline and each injection was administered in a volume of around 200 µl, after adjusting for body weight. The nicotine solution was covered with aluminum foil during storage to prevent light exposure.

DXA scan

Body composition experiments to measure fat depots were carried out by DXA densitometry using a LUNAR PIXImus II Densitometer (GE Medical Systems, New York, NY, USA) with software (version 2.10) and procedures, as described previously (Nagy & Clair 2000) and performed on mice that

were treated with nicotine or saline and HFD or NCD for 10 weeks. Mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) before scanning.

CT scan

CT scans were performed on mice treated with nicotine or saline and receiving HFD or NCD for 10 weeks. Mice received isoflurane (2% by inhalation) anesthesia before imaging in a MicroCAT II small animal CT system (Siemens Preclinical Solutions, Knoxville, TN, USA), as described previously (Suckow & Stout 2008, Suckow *et al.* 2009). A heated anesthesia chamber was used to maintain body temperature and mice were positioned in the center of the field of view. Exposure settings were 70 kVp, 500 mAs, 500 ms exposure time, and 360° rotation in 1° steps with 2.0 mm aluminum filtration. Images were reconstructed using a modified Feldkamp process to a cubic voxel size of 0.20 mm in a 256 × 256 × 496 matrix. CT values were converted into Hounsfield units using the formula: $HU = (\mu_t - \mu_w) / (\mu_w - \mu_a) \times 1000$ where μ_w and μ_a are the linear attenuation coefficients of water and air, respectively, and μ_t is the linear attenuation coefficient of tissue. Using the freely available software AMIDE (<http://amide.sourceforge.net/>), images were analyzed to separate fat (low density) from other soft tissues based on Hounsfield units. The imaging chamber was removed from the CT images using elliptic cylinder region of interest (ROI). The mouse abdominal region was selected from the liver/lung interface to the top of the pelvic horn. An isocontour value selection above -350 Hounsfield units was used to determine the region volume. Adipose tissue volume was selected using isocontour value selection between -268 and -48. Separation of visceral and subcutaneous fat was performed in draw mode by editing the adipose region to contain only one fat type. ROI volumes were calculated using AMIDE and converted to mass assuming an average density of 1.0 g/cm³.

Data analyses

Data are expressed as mean ± S.E.M. Changes in body weight and caloric intake were analyzed using repeated measures ANOVA. DXA scan and CT scan data were analyzed by two-way ANOVA. The Newman–Keuls *post hoc* test was used to reveal significant differences between various treatments. All statistical tests were two tailed and the significance level was set at $P \leq 0.05$.

Results

Nicotine blunted weight gain in mice on NCD and HFD and decreased caloric intake

Figure 1A illustrates the effect of nicotine (1.5 mg/kg per day) vs saline on body weight gain in mice fed with NCD or HFD. Repeated measures ANOVA revealed a significant treatment

Figure 1 Changes in body weight from baseline in mice receiving either nicotine (1.5 mg/kg per day, given twice daily, i.p.) or saline fed with the HFD or NCD (A); in mice on the HFD receiving saline or either high (1.5 mg/kg per day, given twice daily, i.p.), low (0.5 mg/kg per day, given twice daily, i.p.), or escalating dose of nicotine (starting at 0.25 mg/kg per day given twice daily and going up to 4.5 mg/kg per day by an increment of 0.5 mg/kg per day every 3 days, i.p.) (B); and in mice with the NCD receiving either saline or high (1.5 mg/kg per day, given twice daily, i.p.), low (0.5 mg/kg per day, given twice daily, i.p.), or an escalating dose of nicotine (starting at 0.25 mg/kg per day given twice daily and increasing up to 4.5 mg/kg per day by an increment of 0.5 mg/kg per day every 3 days, i.p.) (C). Depicted figure ($n=3-4$ /condition) represents mean ($\pm$ S.E.M.) and is representative of experiment performed on three occasions. * $P < 0.00001$ vs saline/NCD; * $P < 0.00001$ vs saline/HFD.

by time interaction ($F_{141,490}=9.37$; $P<0.000001$). *Post hoc* analysis of the data showed that HFD led to more weight gain than NCD ($P<0.000001$) and nicotine (compared with saline) significantly decreased the weight gain in mice on the NCD ($P<0.000001$) or HFD ($P<0.000001$). The weight-blunting effects of nicotine on the HFD were much more robust than on the NCD. Tolerance to the weight-blunting effects of nicotine did not occur in any of the nicotine-treated groups.

Figure 1B illustrates the effect of low (0.5 mg/kg per day), high (1.5 mg/kg per day), or an escalating dose of nicotine vs saline on changes in body weight in mice fed with the HFD. Repeated measures ANOVA revealed a significant treatment by time interaction ($F_{141,489}=4.08$; $P<0.000001$). *Post hoc* analysis of the data showed a significant decrease in weight gain in mice treated with the high (1.5 mg/kg per day; $P<0.000001$) or escalating ($P<0.000001$) dose of nicotine, but not in those treated with the low (0.5 mg/kg per day) dose of nicotine ($P=NS$) compared with saline-treated controls.

Figure 1C illustrates the effect of low (0.5 mg/kg per day), high (1.5 mg/kg per day), or an escalating dose of nicotine vs saline on changes in body weight in mice fed with the NCD. Repeated measures ANOVA revealed a significant treatment by time interaction ($F_{141,534}=4.08$; $P<0.000001$). *Post hoc* analysis of the data showed that nicotine at high (1.5 mg/kg per day; $P<0.000001$) or escalating dose ($P<0.000001$), but not at the 0.5 mg/kg per day dose ($P=NS$), compared with saline, significantly blunted weight gain induced by the consumption of the NCD. Thus, higher dose nicotine blocks weight gain in mice on both the HFD and the NCD.

Figure 2A shows that the difference in weight gain between mice on nicotine compared to those on saline was accentuated when mice were placed on the HFD after being on the NCD for 45 days. In fact, the saline-treated animals gained about 2 g on the NCD for 45 days and 8 g when placed on the HFD for the next 23 days, whereas the nicotine-treated animals lost about 2 g when fed with the NCD for 45 days and gained only 2 g when placed on the HFD for the next 23 days. Repeated measures ANOVA revealed a significant treatment by time interaction ($F_{26,196}=22.23$; $P<0.000001$).

Figure 2B shows that there was a significant difference in body weight gain in mice on the NCD that were treated with nicotine compared to saline (compare the two groups up to day 23). Repeated measures ANOVA from days 0 to 23 revealed a significant treatment by time interaction ($F_{22,154}=3.61$; $P<0.000001$). Figure 2B also shows that the difference in weight gain between mice receiving nicotine compared with saline was reduced when nicotine treatment was stopped on day 23 (compare the two groups after day 23). Repeated measures ANOVA from days 24 to 38 revealed a significant treatment by time interaction ($F_{15,105}=3.47$; $P<0.0001$).

Figure 3 shows that mecamylamine completely and varenicline partially blocked the weight-reducing action of nicotine in mice fed with the HFD. Repeated measures

Figure 2 Changes in body weight from baseline in mice on the HFD (A) after receiving the NCD for 45 days and given either higher dose nicotine (1.5 mg/kg per day, given twice daily, i.p.) or saline; or (B) in mice on the NCD following nicotine cessation after receiving a higher dose nicotine (1.5 mg/kg per day, given twice daily, i.p.) or saline for 23 days. The data represent mean ($\pm$ S.E.M.) of five mice per time point in each treatment group. * $P<0.00001$ vs saline; ** $P<0.00001$ vs saline for days 1–23; ## $P<0.00001$ vs saline for days 24–38.

ANOVA revealed a significant treatment by time interaction ($F_{65,220}=4.85$; $P<0.000001$). *Post hoc* analysis of the data showed that mecamylamine (nicotine/mecamylamine vs nicotine/saline; $P<0.000001$) and varenicline (nicotine/varenicline vs nicotine/saline; $P<0.00005$) each significantly blunted the weight-reducing action of nicotine. The nicotine/varenicline group was significantly different from the saline/saline group ($P<0.000001$), showing that varenicline only partially blocked the weight-reducing action of nicotine, while there was no difference between the nicotine/mecamylamine group and the saline/saline group ($P=0.27$), indicating a complete inhibition by mecamylamine. *Post hoc* analysis of the data showed that saline/mecamylamine and saline/varenicline groups were not statistically different from the saline/saline group ($P=0.09$ and 0.69 respectively),

Figure 3 Changes in body weight from baseline in mice on an HFD receiving either nicotine (1.5 mg/kg per day, given twice daily, i.p.) or saline with concurrent treatment with either mecamlamine (2 mg/kg per day, given twice daily, i.p.) or varenicline (2 mg/kg per day, given twice daily, i.p.). The data represent mean ($\pm$ S.E.M.) of three to four mice per time point in each treatment group and is representative of data performed on two occasions. * $P < 0.00005$ vs nicotine/saline; # $P < 0.00005$ vs saline/saline.

indicating that mecamlamine and varenicline do not significantly affect body weight by themselves.

Figure 4 illustrates that cumulative caloric intake was mildly reduced by different doses of nicotine compared with saline in mice fed with NCD and HFD, with HFD-treated mice consuming more calories. For NCD, repeated measures ANOVA revealed a significant treatment by time interaction ($F_{141,520} = 1.38$; $P = 0.006$). *Post hoc* analysis of the data showed that nicotine at low (0.5 mg/kg per day; $P = 0.001$) and escalating dose ($P = 0.006$) but not at high dose (1.5 mg/kg per day; $P = 0.96$) significantly decreased the cumulative caloric intake in mice on the NCD. For HFD, cumulative caloric intake was mildly reduced in mice on the HFD treated with different doses of nicotine compared with saline. In HFD-treated mice, the effect of the escalating dose was minimal initially but increased as the dose of nicotine increased. Repeated measures ANOVA revealed a significant treatment by time interaction ($F = 1.66$; $P = 0.0001$). *Post hoc* analysis of the data showed that nicotine at the 1.5 mg/kg per day dose ($P = 0.002$), but not at the escalating ($P = 0.07$) or low (0.5 mg/kg per day; $P = 0.5$) dose significantly decreased caloric intake.

The cumulative caloric intake over the 42 days of the study (Fig. 4) between saline-treated mice and nicotine-treated (1.5 mg/kg per day) mice was 41.2 kcal for mice on the NCD and 48.2 kcal for mice on the HFD. It is estimated that for every 10 kcal reduction in caloric intake, an average mouse loses 1 g of weight (Dalan Jensen, University of Colorado, and Joseph Bass, Northwestern University, personal communication). Thus, the decreased caloric intake possibly accounts for 65% of the reduced weight gain in nicotine-treated (1.5 mg/kg per day) mice on the

HFD (7.3 g less weight gained over the first 42 days, Fig. 1) and all of the reduced weight gain in the NCD (2.6 g less weight gained over the first 42 days, Fig. 1).

Nicotine decreased body fat as determined by DXA and CT scan

Two-way ANOVA revealed a significant effect of diet ($F_{1,12} = 38.8$; $P = 0.00004$), treatment ($F_{1,12} = 6.26$; $P = 0.03$), and interaction between the two factors ($F_{1,12} = 5.03$; $P = 0.04$) for body fat (g) using DXA scan analysis (Fig. 5A). *Post hoc* analysis of the data demonstrated that nicotine (1.5 mg/kg per day) compared with saline decreased the amount of fat ($P < 0.05$) in mice on the HFD, but not in mice fed with NCD ($P = \text{NS}$). As expected, mice on the HFD had a greater amount of fat compared with mice on the NCD ($P < 0.001$).

As shown in Fig. 5B, while two-way ANOVA revealed no significant effect of treatment ($F_{1,12} = 3.04$; $P = 0.11$) on lean mass (muscle plus bone), as measured by DXA, there was a significant effect of diet ($F_{1,12} = 103.6$; $P < 0.0001$) and a significant interaction between the diet and treatment ($F_{1,12} = 11.5$; $P = 0.0053$). *Post hoc* analysis of the data demonstrated that nicotine (1.5 mg/kg per day) compared with saline decreased the amount of lean mass ($P < 0.01$) in mice on the HFD, but not in mice fed with NCD ($P > 0.05$).

Using a two-way ANOVA for the CT scan data for total body fat (Fig. 6A), we found a significant effect of diet ($F_{1,12} = 97$; $P < 0.0001$), indicating that the HFD resulted in higher total fat than NCD, a significant effect of treatment ($F_{1,12} = 5.8$; $P < 0.05$), indicating that the nicotine-treated group had lower total fat than the saline-treated group, and a trend toward a significant interaction between the two factors ($F_{1,12} = 4.4$; $P = 0.08$) showing that nicotine altered the

Figure 4 Cumulative caloric intake in mice fed with the NCD or HFD receiving either saline, high (1.5 mg/kg per day, given twice daily, i.p.), low (0.5 mg/kg per day, given twice daily, i.p.), or an escalating (ESC) dose of nicotine (starting at 0.25 mg/kg per day given twice daily and going up to 4.5 mg/kg per day by an increment of 0.5 mg/kg per day every 3 days, i.p.). The data represent mean ($\pm$ S.E.M.) of three to four mice per time point and is representative of data performed on three separate occasions. * $P < 0.01$ vs saline.

Figure 5 DXA scan showing the total amount of fat (g) (A) and lean mass (muscle plus bone) (B) in mice receiving nicotine (1.5 mg/kg per day, given twice daily, i.p.) or saline fed with the NCD or HFD. * $P < 0.05$ vs HFD-saline group, ** $P < 0.01$ vs HFD-saline group. The data represent mean ($\pm$ S.E.M.) of four mice in each group.

amount of fat more in mice on HFD, although it did not reach significance. *Post hoc* analysis of the data showed that nicotine significantly reduced total fat in the HFD-treated mice ($P < 0.05$), but not in the NCD-treated mice ($P = \text{NS}$). For visceral fat (Fig. 6B), we found a significant effect of diet ($F_{1,12} = 106$; $P < 0.0001$), a significant effect of treatment ($F_{1,12} = 7.2$; $P < 0.05$), and a trend toward a significant interaction between the two factors ($F_{1,12} = 4.4$; $P = 0.06$), showing that nicotine altered the amount of visceral fat more in mice on HFD, although it did not reach significance. *Post hoc* analysis of the data showed that nicotine significantly reduced visceral fat in the HFD-treated mice ($P < 0.05$), but not in the NCD-treated mice ($P = \text{NS}$). For subcutaneous fat (Fig. 6C), we found a significant effect of diet ($F_{1,12} = 72$; $P < 0.0001$), but no significant effect of treatment ($F_{1,12} = 3.1$; $P = \text{NS}$) and no significant interaction between the two factors ($F_{1,12} = 1.95$; $P = \text{NS}$). Thus, nicotine selectively reduced visceral fat, but not subcutaneous fat, in mice on an HFD.

Representative coronal, sagittal, and transverse views of the CT scans of mice on the HFD receiving saline or nicotine (1.5 mg/kg per day) showed a decrease in fat content after combined treatments with HFD plus nicotine compared with HFD plus saline (Fig. 6D).

Discussion

Obesity and cigarette smoking are the leading preventable causes of death in developed societies. Both obesity and cigarette smoking are also important risk factors in many age-related diseases, accelerating the aging process via increasing oxidative stress and inflammation (Valdes *et al.* 2005). Both smoking and consumption of an HFD are risk factors for insulin resistance and type 2 diabetes, and the two habits together can increase the risk of developing these conditions even higher. With an increasing prevalence of obesity, many people report that smoking can be used to control weight gain (Cavallo *et al.* 2010) and adolescent smokers frequently engage in unhealthy dietary restrictions (fasting, diet pills, or vomiting) (Cavallo *et al.* 2010). Once they start smoking, some people may develop an erroneous belief that they can eat unhealthy diets. Thus, understanding the combined effects of nicotine, the main ingredient in cigarettes that affects body weight (Schechter & Cook 1976, Grunberg 1982) and body fat distribution has important public health consequences.

Our study showed that nicotine reduced weight gain in mice, an effect that was much more pronounced in mice fed with the HFD compared with those on the NCD. The effect was more robust at the higher dose of nicotine. We also found that when mice were switched from an NCD to HFD, the weight loss properties of nicotine were accentuated. Furthermore, nicotine cessation led to weight gain and therefore a reduction in the difference observed on weight gain between nicotine- and saline-treated mice on the NCD. The accelerated weight gain following nicotine cessation is consistent with previous animal (Schechter & Cook 1976, Grunberg *et al.* 1984, 1985, 1986, Winders & Grunberg 1990) and human (Grunberg 1982, Grunberg & Morse 1984) studies, although other studies reported that body weight remained attenuated following smoking cessation (Bellinger *et al.* 2010). This result may also suggest that the weight-reducing action of nicotine is dependent on nicotine intake and may involve some rapidly reversible processes that may be turned off following the cessation of nicotine treatment.

The nicotine-induced changes in body weight may be due to decreased energy intake, increased energy expenditure, or a combination of the two processes. The literature provides support for decreased food intake (Munster & Battig 1975, Grunberg 1982, Clarke & Kumar 1984, Grunberg *et al.* 1985, Chen *et al.* 2007b), but there is also some evidence showing no effect on food intake (Schechter & Cook 1976, Grunberg *et al.* 1984), especially in male rats (Grunberg *et al.* 1987, Winders & Grunberg 1990). A recent article found that nicotine, given to mice on an HFD, decreased food intake

Figure 6 CT scans in mice receiving nicotine (1.5 mg/kg per day, given twice daily, i.p.) or saline and fed with the NCD or HFD showing total fat (A), visceral fat (B), and subcutaneous fat (C), and representative view of visceral and subcutaneous fat using coronal, sagittal, and transverse sections (D). The data represent mean ($\pm$ S.E.M.) of four mice per group. * $P < 0.05$ vs saline.

and increased energy expenditure, while nicotine withdrawal resulted in weight gain due to a decrease in energy expenditure (Hur *et al.* 2010). The relative effect of food intake vs energy expenditure may have to do with species, gender, type of food (bland vs sweet food), time of nicotine exposure (light or dark cycle), method of nicotine administration (pump vs injections), or dose of nicotine. Additional studies demonstrated increased metabolism in rodents exposed to nicotine (Bizzi *et al.* 1972, Ilebek *et al.* 1975, Bellinger *et al.* 2010). We found that caloric intake was decreased in nicotine-treated mice. Estimates of energy intake suggested that decreased caloric intake accounted for all the weight loss in mice on an NCD and 66% on an HFD. This suggests that some of the weight loss in mice on an HFD may be due to increased energy expenditure. It is worth noting that the conversion between reduced caloric intake and decreased body weight is only an estimate.

In both humans and rats, nicotine administration or cigarette smoking has been shown to selectively depress the consumption of sweet-tasting high-caloric foods (Grunberg & Morse 1984, Grunberg *et al.* 1985). The hypophagic and weight-reducing actions of nicotine in rats depend mainly on the sweet taste rather than the caloric content of the food, when these two variables are independently manipulated (Grunberg *et al.* 1985). Female rats are more sensitive to the weight-reducing effects of nicotine than male rats (Grunberg *et al.* 1986). Additionally, female rats gain more weight following smoking cessation than male rats (Grunberg *et al.* 1986). Thus, future work is needed to examine whether the present results extend to female mice.

Our studies as well as most studies looking at weight-reducing effects of nicotine used nicotine and not cigarette smoke. Chen *et al.* (2007a), however, found that cigarette smoke exposure reduced body weight more substantially in Balb/c mice on a regular chow compared with mice on the HFD. In that study, smoke exposure reduced weight gain without significantly reducing fat mass and the authors found a reduction in food intake with smoke exposure.

An important issue that our study begins to address is which nAChR mediates the nicotine-induced weight loss and change in body composition. nAChRs are divided into muscle ($\alpha 1$, $\beta 1$, γ/ϵ , and δ) and neuronal subtypes ($\alpha 2$ –10 and $\beta 2$ –4) (Dani 2001). In addition, nonneuronal cells express functional nAChR, including keratinocytes, bronchial epithelial cells and aortic endothelial cells, and lymphocytes (Maslinski *et al.* 1992, Nguyen *et al.* 2000, Liu *et al.* 2004). Recently, Liu *et al.* (2004) found that rat adipocytes express $\alpha 1$ –7, $\alpha 9$, $\alpha 10$, $\beta 1$ –4, δ , and ϵ subunit mRNA. Immunocytochemical analysis also suggested the presence of $\alpha 7$ and $\beta 2$ subunits in adipocytes and receptor binding studies showed high-affinity binding for nicotine in adipocytes (Liu *et al.* 2004).

Our finding that mecamylamine completely blocked the effects of nicotine implies that nicotine receptors are involved, although we are unable to determine whether the effect is mediated via the central or peripheral nAChRs. In the future, we may perform experiments with antagonists that do not cross the blood–brain barrier to help determine whether the effects are mediated via the central or peripheral nAChRs. It is possible that nicotine is having a direct effect on the

adipocyte, which expresses nAChRs as discussed above, or may be an effect at sites related to increased metabolism, such as via the adrenal medulla where nAChR activation may lead to increased catecholamine release (Sala *et al.* 2008) or brown fat, where nAChR activation may stimulate expression of uncoupling proteins 1 and 3 promoting thermogenesis (Chen *et al.* 2008).

Our study also examined the effects of varenicline, an $\alpha 4\beta 2$ nAChR partial agonist/antagonist that is used clinically for smoking cessation under the trade name of Chantix (Gonzales *et al.* 2006, Siu & Tyndale 2007) on nicotine-induced weight loss. Varenicline partially blocked the nicotine-induced weight loss, suggesting that $\alpha 4\beta 2$ nAChR is partially involved in the weight-reducing properties of nicotine, but other nAChRs are also likely to be involved. None of the antagonists/partial agonists altered body weight in mice given saline, although a trend toward weight gain was seen with saline plus varenicline. Clinically, weight gain has been found with varenicline (Physicians' Desk Reference 2011. <http://www.pdr.net/>, Accessed October 26, 2011), although this may be due to the weight gain observed following smoking cessation.

Smokers are leaner yet paradoxically have increased rates of cardiovascular disease and type 2 diabetes. This may be explained by the type of fat distribution in smokers. Visceral fat is known to be more detrimental in terms of cardiovascular disease, diabetes, and overall mortality (Lapidus *et al.* 1984, Larsson *et al.* 1984, Sparrow *et al.* 1986, Fujioka *et al.* 1987). Smokers have an elevated risk of having a high waist-hip ratio (WHR) with increased central obesity/visceral fat even with a low BMI (Moffatt & Owens 1991, Jee *et al.* 2002) and a graded dose-response relationship was found between the number of cigarettes smoked and WHR (Shimokata *et al.* 1989). The WHR in those who started smoking actually increased despite their loss of weight. These paradoxical changes in WHR indicate that there are harmful effects of cigarette smoking on the pattern of distribution of body fat (Shimokata *et al.* 1989). However, other studies have found that smokers have a lower amount of visceral fat than nonsmokers (Matsushita *et al.* 2010). Because of the effect of cigarettes on fat distribution in humans, we determined how nicotine affects fat distribution in mice fed with the HFD compared with the NCD. We hypothesized that we would see an increase in the amount of visceral fat in nicotine-treated mice, similar to subjects using cigarettes. DXA scan showed that nicotine did not alter the amount of fat in mice on the NCD but did substantially reduce the amount of fat in mice fed with the HFD. CT scan analysis confirmed that nicotine led to a substantial reduction in fat stores, but only in mice on the HFD diet. Surprisingly, nicotine treatment resulted in a decreased amount of visceral fat in mice on HFD, which is similar to the above study (Matsushita *et al.* 2010). Thus, the more detrimental fat appears to be selectively reduced by nicotine.

Our DXA scan analysis demonstrated a decrease in both fat and lean body mass in mice receiving HFD and treated with nicotine compared with their saline-treated controls.

The reduction in fat mass is expected and is consistent with a decreased body weight found in this group. The decreased lean mass quantitated by DXA may be due to ectopic fat deposition in the muscle of mice treated with nicotine/HFD, as we have reported in a preliminary study (Shin *et al.* 2011). Our DXA finding showing a lack of an effect of nicotine on both fat and lean body mass in mice receiving NCD might be due to the small overall differences in weight between the two groups that might not be detected by DXA scan.

Our study has some limitations. Nicotine was delivered by twice daily i.p. injections. We recognize that this system does not incorporate voluntary consumption and also that only twice a day peak of nicotine is nonphysiological, compared with how smokers receive nicotine. However, other models have their own drawbacks. For example, Alzet pump to deliver nicotine or saline continuously is not clinically relevant either and our unpublished data demonstrate that it can lead to increased corticosterone levels due to the postoperative pain and distress associated with osmotic minipump implantation. Thus, we are confident that this model is a realistic choice to study the effects of nicotine on body weight and fat redistribution.

In conclusion, we observed that nicotine exposure reduced HFD-induced weight gain and further led to lower amounts of body fat and a more favorable distribution of body fat (a trend toward more subcutaneous and less visceral fat). Thus, superficially, it might appear that the HFD plus nicotine is actually a more beneficial combination than HFD alone. However, several recent animal studies have shown that cigarette smoking exacerbates hepatic steatosis triggered by HFD (Liu *et al.* 2003, Chen *et al.* 2007a, Yuan *et al.* 2009, Azzalini *et al.* 2010). In preliminary studies, our laboratory has found that nicotine plus the HFD leads to impaired glucose tolerance (Friedman *et al.* 2009), increased oxidative stress, and hepatic and muscle steatosis (Mangubat *et al.* 2011). Therefore, we certainly do not advocate smoking or nicotine use to combat the untoward consequences of consumption of the HFD as it is likely that these two insults can act synergistically to exhibit severely detrimental metabolic effects, more than each insult alone.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. None of the authors has a relationship with the tobacco industry and no funding from any tobacco company was received for this work.

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