The effect of short term cigarette smoke exposure on body weight, appetite and brain neuropeptide Y in mice

Hui Chen[1], Ross Vlahos[2, 3], Steve Bozinovski[1, 3], Jessica Jones[1, 3], Gary P. Anderson[1, 2, 3], Margaret J. Morris[1].

1. Department of Pharmacology, The University of Melbourne, Victoria 3010, Australia.

2. Department of Medicine, The University of Melbourne, Royal Melbourne Hospital, Victoria 3050, Australia.

3. CRC for Chronic Inflammatory Diseases, The University of Melbourne, Victoria 3010, Australia.

Corresponding author:

Assoc Prof. Margaret J. Morris

Department of Pharmacology, The University of Melbourne

Vic 3010, Australia.

Telephone: 61 (3) 8344 5745

Fax: 61 (3) 8344 0241

Email: mjmorris@unimelb.edu.au

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Abstract

Although nicotinic receptors have been demonstrated in hypothalamic appetite regulating areas and nicotine administration alters food intake and body weight in both animals and humans, the mechanisms underlying the effects of smoking on appetite circuits remain unclear. Conflicting effects of nicotine on the major orexigenic peptide, neuropeptide Y (NPY), have been observed in the brain, but the effects of smoking are unknown. Thus we aimed to investigate how cigarette smoking affects body weight, food intake, plasma leptin concentration, hypothalamic NPY peptide, adipose mass and mRNA expression of uncoupling proteins (UCP) and TNF α . Balb/c mice (8 weeks) were exposed to cigarette smoke (3 cigarettes, 3 times a day for 4 consecutive days) or sham exposed. Body weight and food intake were recorded. Plasma leptin and brain NPY were measured by radioimmunoassay. UCPs and TNF α mRNA were measured by real time PCR. Food intake dropped significantly from the first day of smoking, and weight loss became evident within 2 days. Brown fat and retroperitoneal white fat masses were significantly reduced, and plasma leptin concentration was decreased by 34%, in line with the decreased fat mass. NPY concentrations in hypothalamic subregions were similar between two groups. UCP 1 mRNA was decreased in white fat and UCP 3 mRNA increased in brown fat in smoking group. Short term cigarette smoke exposure led to reduced body weight, food intake and fat mass. The reduction in plasma leptin concentration may have been too modest to increase NPY production; alternatively, change in NPY or its function might have been offset by nicotine or other elements in cigarette smoke.

Key words:

anorexia, weight loss, cigarette smoking, adipose tissue, uncoupling protein, tumor necrosis factor α

Introduction

An inverse relationship between cigarette smoking and body weight has been documented in many studies. Smokers weigh less than non-smokers of the same age and gender, and anorexia often occurs with cigarette smoking (Albanes et al. 1987; Klesges et al. 1989; Perkins 1992). Cessation of smoking without nicotine replacement therapy is usually accompanied by weight gain (Stamford et al. 1986; Levin et al. 1987; Williamson et al. 1991; O'Hara et al. 1998). As a consequence, some people are willing to use smoking in order to lose or maintain their body weight, and are reluctant to stop smoking due to concerns about weight gain on cessation, especially among younger smokers (Crisp et al. 1999; Wee et al. 2001). The effects of cigarette smoking on appetite may also contribute to the morbidity and mortality of smokers with chronic lung disease and wasting.

This action of smoking on body weight appears to be nicotine mediated as indicated by Hajek and colleagues (Hajek et al. 1988). Previous studies in both humans and animals have reported that nicotine administration decreases body weight and caloric intake (Wager-srdar et al. 1984; Grunberg et al. 1986; Hajek et al. 1988; Bellinger et al. 2003; Bishop et al. 2004). Thus the effects of nicotine to suppress appetite and decrease food intake leading to reduced body weight are not confined to human subjects.

The brain responds to altered energy homeostasis by adjusting food intake. Neuropeptides in the

hypothalamus play a pivotal role in regulating food intake, and their actions in this region are known to be modulated by circulating factors, such as leptin. Neuropeptide Y (NPY), a 36-amino acid neuropeptide which is abundant in the mammalian brain and highly concentrated in the hypothalamus, is known to play a central role in hypothalamic mechanisms which increase food intake (Woods et al. 1998). Nicotine binding sites have been demonstrated in appetite regulating regions of the hypothalamus suggesting that centrally mediated actions of nicotine may contribute to the reduced appetite and body weight loss (Jo et al. 2002). If NPY signaling is a possible target for nicotine's anorexic effects, a suppression of NPY expression might be expected with nicotine treatment. Frankish and colleagues found that both acute (24 hour) and longer term (2 week) nicotine injection decreased NPY content and NPY mRNA expression in the arcuate nucleus and paraventricular nucleus of the rat, however Li and colleagues have shown a positive correlation between NPY mRNA expression and the dose of nicotine used (Frankish et al. 1995; Li et al. 2000). Thus the picture regarding the effects of nicotine on NPY in the central nervous system is unclear. Further the effects of smoking, rather than nicotine, on brain mediators involved in appetite regulation, have not been investigated to date.

In addition to examining NPY, we also investigated the effects of smoking on other markers of obesity and energy homeostasis, such as leptin and uncoupling proteins (UCP). Leptin is a hormone secreted by adipose tissue that is known to act centrally to inhibit the effects of NPY, apparently by inhibiting its synthesis in the arcuate nucleus (Stephens et al. 1995; Cusin et al.

1996; Erickson et al. 1996; Elmquist et al. 1999). The distribution of leptin receptors was found to overlap with that of nicotinic receptors in the hypothalamus, indicating a possible link between these two factors (Jo et al. 2002). Uncoupling proteins (UCPs) are mitochondrial carrier proteins which are able to dissipate the proton gradient of the inner mitochondrial membrane. This uncoupling process reduces the amount of ATP generated through oxidation of fuels and increases thermogenesis, which is related to energy metabolism (Dalgaard et al. 2001).

Smoking exerts an inflammatory stimulus on macrophages which brings about the production of inflammatory cytokines, such as tumor necrosis factor (TNF) α , which might be an important early event in the development of disease states associated with smoking (Fernandez-Real et al. 2003). Increased TNF α activity (Fernandez-Real et al. 2003) and decreased actions have been observed in smokers and nicotine administration studies (Ouyang et al. 2000; Liu et al. 2001). Produced by both immunocompetent cells and adipocytes, TNF α can regulate lipid metabolism, adipocyte differentiation, inhibit appetite, and is a mediator of cachexia (Torti et al. 1989; Spiegelman and Hotamisligil 1993; Ventre et al. 1997; Bullo-Bonet et al. 1999; Langhans and Hrupka 1999). Therefore we also determined the effect of cigarette smoke exposure on TNF α expression in adipose tissue.

Therefore the aim of this study was to measure the changes in food intake, body weight, hypothalamic NPY content and expression of UCP1, UCP3 and TNF α mRNA in fat tissue in

response to short term (4 days) cigarette smoke exposure in mice.

Materials and Methods

1. Animals

Development of respiratory disease following cigarette smoke exposure is strain dependent (Guerassimov et al. 2004), and in this study Balb/C mice were selected based on the inflammatory and cytokine responses in related respiratory studies in our laboratory. Male Balb/C mice (aged 7 weeks, n = 32) were obtained from the Animal Resource Centre Pty. Ltd. (Perth, Australia), and were housed at 20 ± 2℃ in micro-isolator cages, and maintained on a 12:12 h light/dark cycle (lights on at 06:00 h). They were allowed *ad libitum* access to standard rodent chow and water. Mice were allowed a week to adapt to their new environment. Animals were monitored daily; food intake per cage and individual body weight were measured 3 times per week during this period. After acclimatization mice were randomly divided into two groups, cigarette smoke exposed (smoking group) or sham exposed (control group). The animals exposed to cigarette smoke were placed inside a perspex chamber (18 liters) and exposed to the smoke produced by 3 cigarettes (Winfield Red, 16 mg or less of tar, 1.2 mg or less of nicotine and 15 mg or less of CO), 3 times (09:00, 12:00 and 15:00 h) a day for 4 consecutive days. Control mice were handled similarly without smoke exposure. Food intake and body weight were measured daily. All procedures were approved by the Animal Experimentation Ethics Committee of The University of Melbourne.

2. Sample collection

On the 5th day, mice were anesthetized (ketamine/xylazine 15/30 mg/kg, intraperitoneal), and blood was collected from the abdominal vena cava into heparinised tubes (5000 IU/ ml; Fisons, Australia), and centrifuged at 10,000 r.p.m. and 4℃ for 8 minutes. Separated plasma was stored at -80℃ for subsequent determination of plasma leptin and corticosterone concentrations. Mice were decapitated and the brain removed and rapidly dissected on ice into regions containing paraventricular nucleus (PVN), arcuate nucleus (Arc), anterior and posterior hypothalamus (AH and PH), and medulla. Brain regions were weighed and stored at -80℃ for later determination of NPY peptide content. Body fat (brown adipose tissue (BAT), left retroperitoneal white adipose tissue (RpWAT), testicular WAT) and liver were dissected and weighed. BAT and RpWAT were stored at -80 ℃ for later measurement of mRNA of UCP1, UCP3 and TNF α.

3. Brain NPY, plasma leptin and corticosterone assays

Endogenous NPY from the various brain regions was extracted by boiling the tissues in 0.5 M acetic acid, homogenisation by hand with a glass homogeniser, and centrifugation at 7500 r.p.m. for 30 minutes at 4 ℃. The supernatant was decanted, and 50 µl samples were lyophilized and reconstituted with assay buffer (0.04 M sodium phosphate buffer containing 0.01 M EDTA, 0.1 M NaCl, 0.02% NaN_3, 0.25% BSA, pH 7.3). NPY-like immunoreactivity in the various brain regions was measured by a specific radioimmunoassay developed in our laboratory using

synthetic NPY as standard (10-1280 pg/tube, Auspep, Australia) (Morris et al. 1986). Samples were incubated with NPY antibody overnight at 4℃. ^{125}I-NPY labeled with Bolton and Hunter reagent (3000 Ci/mmol, Amersham, Australia) was added and the incubation continued overnight. Bound and free radioligand were separated by the addition of non-immunized rabbit serum and sheep anti-rabbit second antibody followed by centrifugation at 3000 r.p.m. at 4℃ for 35 minutes (RT7, Sorvall instruments). The bound fraction was then counted in a gamma counter. The detection limit for the radioimmunoassay was routinely 2 pg NPY per tube and the intra- and inter-assay coefficients of variation were 6% and 13% respectively. NPY in each brain region was calculated as ng NPY per mg tissue. Plasma leptin and corticosterone concentrations were measured using a commercially available radioimmunoassay kit (Linco, Missouri, USA, and MP Biomedicals Europe, Belgium, respectively).

4. UCP1, UCP3 and TNF α measurement

Total RNA was isolated from 10 mg of both WAT and BAT using a RNAeasy kit (Qiagen) according to manufacturer instructions. The purified total RNA rep was used as a template to generate first-strand cDNA synthesis using SuperScript II (Invitrogen) as previously described (Bozinovski et al. 2004). The reaction mix containing 1μg of RNA, 250 ng of random hexamers (Promega) and 10 mM dNTP mix was diluted to 12 μl in sterile water, heated to 65ºC for 5 minutes and chilled on ice for 1 minute. First strand synthesis was then performed in a 20 μl total reaction volume by adding 50 mM Tris.HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT,

40 units RNaseout and 200 units Superscript II reverse transcriptase enzyme at 42ºC for 50 minutes. The reaction was inactivated by heating at 70ºC for 15 minutes. cDNA was diluted 10-fold in sterile water and stored at -20ºC prior to amplification. Quantitative real time PCR was performed as previously described (ABI PRISM 7900 HT Sequence Detection System) (Bozinovski et al. 2004) using predeveloped primers from Applied Biosystem. Briefly, gene expression was quantified by multiplexing single reaction, where our gene of interest (UCP1, UCP3 and TNF α) was standardized to control (18s rRNA). An individual BAT sample from the control group was then arbitrarily assigned as a calibrator against which all other samples are expressed as a fold difference.

5. Statistical analyses

Results are expressed as mean ± S.E.M. Body weight of smoke exposed and control mice were analyzed using Analysis of Variance (ANOVA) with repeated measures, followed by a post hoc least significance difference (LSD) test. Difference in daily food intake, fat and organ mass, plasma leptin and corticosterone concentrations, brain NPY concentration and content, and relative expression of mRNA were analyzed using *Student's* unpaired *t*-tests. The relation between plasma leptin concentration and body weight, body weight gain and WAT was examined using simple linear regression.

Results

Both food intake and body weight of the two experimental mice groups were well matched at the beginning of this study as shown by Fig. 1a and Fig. 1b respectively. Over the whole experimental period, daily food intake of control mice was stable. After the first day of cigarette smoke exposure, food intake was somewhat lower than control mice, and this difference increased over time. From the second day of smoke exposure, food intake was reduced to 60% of the control level (Fig. 1a, $P < 0.05$). The total 4 day chow intake of the mice exposed to cigarette smoke was reduced by 34% (9.4 vs. 14.3 g/mouse in control mice, $P < 0.05$). There was a time effect on the growth of control mice, reflecting weight gain, which was opposite in the smoking group ($P < 0.05$, Fig. 1b). Body weight of the smoking group was significantly reduced within 2 days of cigarette smoking (21.9 ± 0.3 g vs. 23.6 ± 0.3 g for smoking and control group respectively, $P < 0.05$, Fig. 1b) and pre-treatment (day 7) levels ($P < 0.05$, Fig. 1b). After 4 days of cigarette smoke exposure, the average body weight of the smoking group was 10% less than the control group ($P < 0.05$, Table 1).

Animals exposed to 4 days of cigarette smoke displayed lower weights of liver, BAT and RpWAT relative to control mice ($P< 0.05$, Table 1). Notably for BAT and RpWAT these differences were still pronounced when data were calculated as a percentage of body weight (BAT 0.38 ± 0.02% vs. 0.47 ± 0.02%; RpWAT 0.48 ± 0.04% vs. 0.59 ± 0.03%, in smoking and control group respectively, $P < 0.05$). Plasma leptin concentrations in the smoking group decreased significantly, in line with the decreased white fat mass ($P < 0.05$, Table 1). When both groups are combined

plasma leptin concentration was significantly correlated with WAT, body weight and body weight gain ($r = 0.60$, 0.54 and 0.58 respectively, $P < 0.05$, $n = 15$). No difference in plasma corticosterone levels was observed between treatment groups at the end of the experiment (Table 1).

NPY concentrations of the hypothalamic regions, AH, PH, PVN, and Arc, as well as medulla, were not different between the control and the smoking groups at the end of the experiment (Fig. 2). The total hypothalamic NPY content was not altered by 4 days smoke exposure (smoking group 82.3 ± 1.9 ng vs. control group 86.2 ± 2.9 ng).

UCP1 mRNA expression was decreased by 76% in WAT in the smoking group, but no difference was found in BAT between the smoking and control group**.** BAT UCP3 mRNA expression was significantly increased in cigarette smoke exposed mice, while no change was observed in WAT. TNF α mRNA expression was not affected by cigarette smoking in either WAT or BAT (Fig. 3).

Discussion

In this study, we examined the hormonal, neurochemical and metabolic changes induced by short term (4 consecutive days) cigarette smoke exposure in mice. Using this protocol we previously determined blood carboxyhemoglobin content to be 3% (unpublished observation), suggesting a moderate level of smoke exposure in the smoking group, compared to 10-14% in heavy smokers

(Benowitz et al. 1982). When exposed to cigarette smoke, the mice showed a consistent reduction in chow intake compared to both their baseline levels before cigarette smoke exposure was implemented, and the chow intake of the control group. The unchanged levels of plasma corticosterone, a stress response hormone, between experimental groups, suggests decreased food intake might not be due to any greater stress related to smoke exposure. This reduced appetite with smoke exposure is consistent with the anorexia commonly observed among human smokers and previous studies using nicotine administration (Grunberg et al. 1986; Albanes et al. 1987; Levin et al. 1987; Hajek et al. 1988; Klesges et al. 1989; Bellinger et al. 2003; Bishop et al. 2004). However clearly it is difficult to draw comparisons between the experiments using nicotine administration and effects of cigarette smoke, which contains multiple factors, and can cause inflammatory changes which might alter energy metabolism. The decreased food intake most likely led to the significant reduction in body weight, which was 10% less than control mice at death, reflected by a significant reduction in adipose tissue, particularly white fat masses. When data were calculated as a percentage of body weight, white fat masses remained significantly reduced in the smoking group, indicating decreased body fat largely contributed to the reduced body weight, and suggesting fat deposits might be used as an energy supply under these conditions of negative energy balance. The anorexia and weight loss caused by cigarette smoke exposure in mice appears to resemble the effects of cigarette smoking in humans.

NPY is synthesized in the Arc, from where cells project to the PVN and other hypothalamic areas

(Bai et al. 1985; Chronwall et al. 1985). Exogenous administration of NPY into the cerebroventricle results in a dramatically increased carbohydrate and fat intake and decreased energy expenditure in rodents, and eventually, to a state of obesity (Zarjevski et al. 1993). NPY peptide production is increased with starvation, food restriction and in genetically obese animals (eg. *ob/ob* mice), and decreased with refeeding, suggesting NPY may be a critical mediator for the maintenance of body weight (Beck et al. 1990; Brady et al. 1990; Wilding et al. 1993; Swart et al. 2002). Since nicotinic receptors have been demonstrated in the appetite regulating area of the hypothalamus, cigarette smoking might change appetite in mice by affecting the energy homeostasis circuits including both those releasing orexigenic peptides, such as NPY and melanin concentrating hormone, and anorexigenic peptides, such as cocaine- and amphetamine-regulated transcript and pro-opiomelanocortin (Jo et al. 2002). However, NPY concentrations in the hypothalamic subregions, AH, PVN, PH and Arc, as well as the medulla, were not affected by 4 days of smoke exposure. In previous nicotine administration studies in rat, decreased NPY peptide in PVN and Arc was observed after 24 hours of high dose (12 mg/kg/day) nicotine administration that reduced food intake by 30% (Frankish et al. 1995), while food intake of mice exposed to cigarette smoke in the present study was only 16% less than control mice in the first 24 hours, suggesting the extent of negative energy status induced by the cigarette smoke exposure we used might be insufficient to regulate hypothalamic NPY. However in another study hypothalamic NPY peptide was found to be increased after 14 days of low dose (4 mg/kg/day) nicotine treatment which reduced food intake by 19.5% in rats (Li et al. 2000), which suggests

the present treatment may have been too short to cause significant changes in NPY peptide. Furthermore, previously it was found that nicotine can directly inhibit the hyperphagia produced by exogenous administration of NPY into the PVN (Bishop et al. 2002), suggesting it is possible that the effects of brain NPY can be offset by nicotine or other elements in cigarette smoke. The effects of nicotine administration on NPY peptide in mice have not been documented previously. Decreased leptin levels would normally decrease the inhibitory effects of leptin on NPY and thus increase NPY production (Stephens et al. 1995; Elmquist et al. 1999). Although we have previously described significant negative correlations between plasma leptin and hypothalamic NPY in a number of paradigms including weight loss following vagotomy (Furness et al. 2001), and chronic high fat diet (Hansen et al., 2004), the impact of cigarette smoke exposure on leptin here was relatively modest compared to these interventions.

In addition to the decreased food intake, it is possible that other factors involved in energy balance may also have contributed to the decreased body weight observed in mice exposed to cigarette smoke. While it is generally accepted that UCP 1 is exclusively expressed in BAT in rodents, it can be induced in WAT at a lower levels than in BAT (Nagase et al. 1996; Yoshida et al. 1999). In our study, UCP 1 in BAT was not affected by 4 days smoke exposure, suggesting BAT thermogenesis was largely unaffected by cigarette smoking in this protocol. By using quantitative real time PCR, UCP 1 mRNA can be detected under basal conditions in WAT in mice. The expression of UCP 1 mRNA in WAT was found to be significantly decreased by cigarette smoke

exposure. However previously it was reported that UCP 1 mRNA can be induced in both BAT and WAT by nicotine treatment, probably enhancing the energy expenditure in this situation (Yoshida et al. 1999; Arai et al. 2001). These changes highlight the need to examine metabolic effects of smoking per se rather than nicotine administration. Fasting and chronic food deprivation can downregulate UCP 1 expression in BAT (Samec et al. 1998; Sivitz et al. 1999), with changes in WAT unknown. Whereas, in our study a 34% reduction of total food intake over 4 days cigarette smoke exposure did not affect UCP 1 mRNA expression in BAT, but reduced it in WAT. Although overexpression of UCP 1 mRNA in WAT can cause obesity resistance (Kopecky et al. 1996), it is not clear how the decreased UCP 1 mRNA might contribute to the maintenance of energy balance in this situation. It is difficult to separate any effects of nicotine or food restriction on UCP1, as both have been shown to regulate expression of UCPs.

UCP 3, a homolog of UCP 1, is expressed in BAT and skeletal muscle and is implicated in the regulation of mitochondrial fatty acid transport and influences basal metabolic rate (Samec et al. 1998). In our study, UCP 3 mRNA was significantly increased in BAT in the face of the reduced food intake following smoke exposure, which was opposite to previous studies where the UCP 3 mRNA expression in BAT was downregulated by fasting and food deprivation, a physiological response to conserve energy expenditure (Samec et al. 1998; Sivitz et al. 1999). The role of UCP 3 in lipid regulation in skeletal muscle can be extended to BAT (Samec et al. 1998). An increase in UCP 3 would increase uncoupling of mitochondrial respiration and increase energy

expenditure or heat dissipation, which suggests in the smoke exposed mice, lipid utilization and energy expenditure were upregulated, rather than decreasing energy expenditure to maintain homeostasis. Thus cigarette smoke exposure probably disturbs energy homeostasis, which may crucially contribute to the weight and adipose loss observed. Very little data exists on the effects of nicotine administration on BAT UCP 3 mRNA expression in rodents to date.

TNF α is increasingly recognized to be involved in the pathogenesis of obesity, and its activation seems to be associated with increased energy expenditure and weight loss (Toomey et al. 1995; Tracey and Cerami 1992). Previously it was found that TNF α is increased in patients with anorexia (Vaisman and Hahn 1991), and exogenous administration of TNF α had inhibitory effects on food intake in rats (Fantino and Wieteska 1993; Sonti et al. 1996; McCarthy 2000). In addition, TNF α can affect lipid metabolism *in vivo* by increasing lipolysis in adipose tissue, and inhibit adipose cell differentiation *in vitro* (Torti at al. 1989; De clercq et al. 1996; Kern 1997). However our observation of no change in TNF α mRNA expression in both BAT and WAT suggests that TNF α (at least in adipose tissue) did not contribute to anorexia and the loss of fat deposits following cigarette smoke exposure.

In summary, 4 days cigarette smoking caused loss of appetite and body weight in mice, and accordingly negative energy balance, but increased lipid utilization and energy expenditure reflected by an induced UCP 3 mRNA expression in BAT. Hypothalamic NPY concentration and

content were not changed by 4 days cigarette smoke exposure, probably due to the short term nature of this experiment or the inhibitory effects of cigarette smoke exposure. Further work is underway to determine effects of more chronic periods of cigarette smoking.

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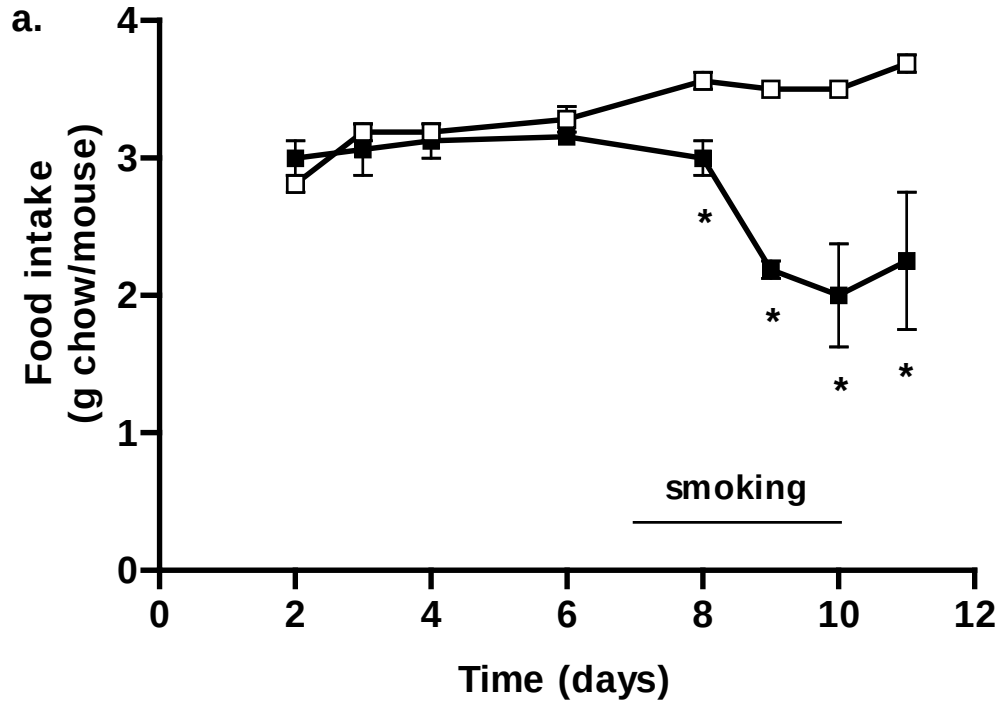
Table 1: Effect of cigarette smoke exposure on body weight, liver, adipose tissue mass, plasma leptin and corticosterone concentration.

	Control	*Smoke exposure*
Body weight (g) (pre-exposure)	23.0 ± 0.3	22.5 ± 0.2
Body weight (g) (post-exposure)	23.6 ± 0.3	21.3 ± 0.4*
Liver (mg)	1188.5 ± 38.5	929.8 ± 20.3*
BAT (mg)	110.7 ± 6.5	80.0 ± 4.0*
RpWAT (mg)	139.1 ± 7.7	104.3 ± 9.7*
Testicular WAT (mg)	471.7 ± 24.9	416.4 ± 34.9*
Leptin (ng/ml)	6.5 ± 0.5	4.3 ± 0.5*
Corticosterone (ng/ml)	212.4 ± 50.7	247.3 ± 33.6

Results are expressed as mean ± S.E.M (for body weights, n = 32; for tissue masses, n = 11, 12 respectively; for plasma hormone concentrations, n = 8, 7 respectively).

* Significantly different from control mice ($P < 0.05$).


a.
Food intake
(g chow/mouse)
4
3
2
1
0
0
2
4
6
8
10
12
Time (days)
smoking
*
*
*
*

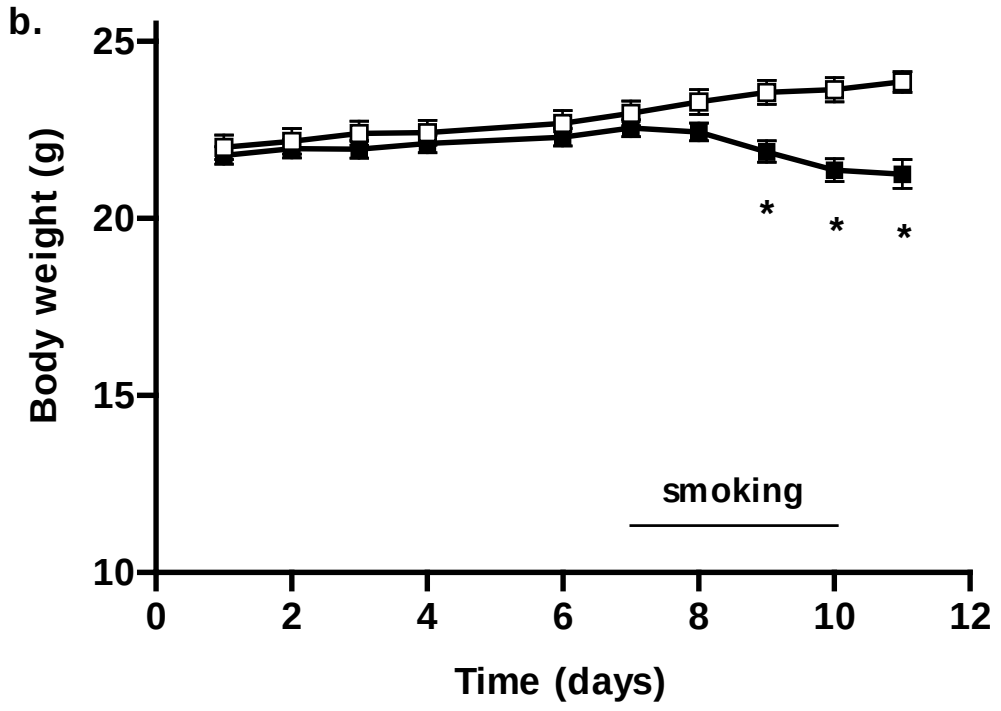

b.
Body weight (g)
25
20
15
10
0
2
4
6
8
10
12
Time (days)
smoking
*
*
*

Fig. 1

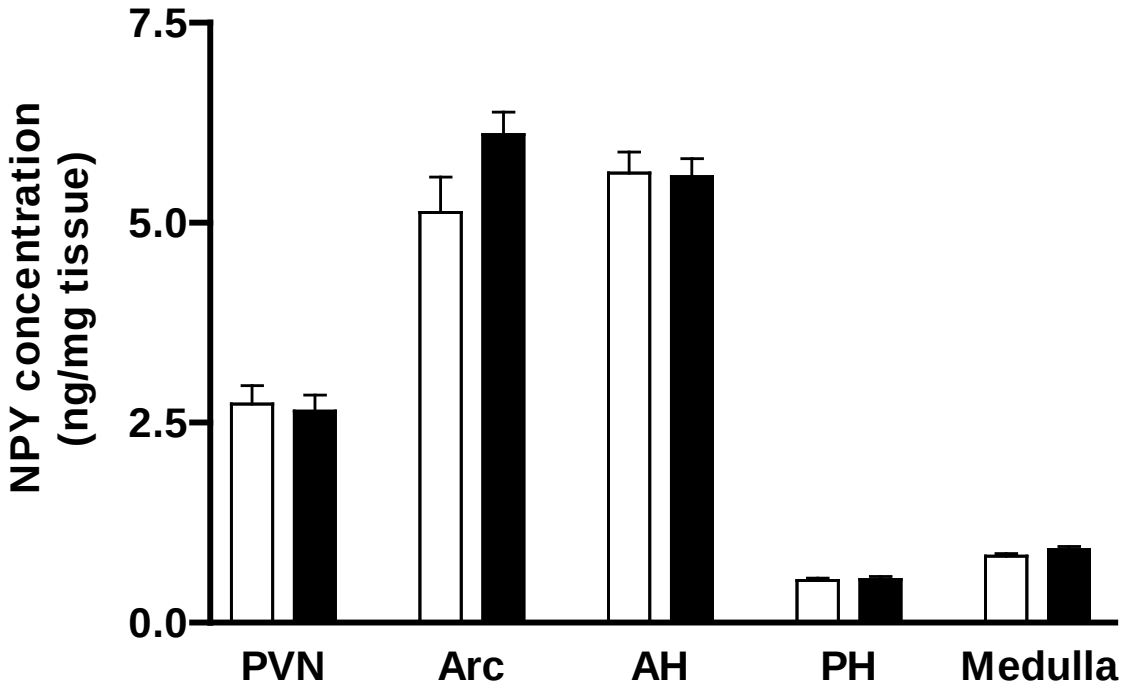

7.5
5.0
2.5
0.0
NPY concentration
(ng/mg tissue)
PVN
Arc
AH
PH
Medulla

Fig. 2

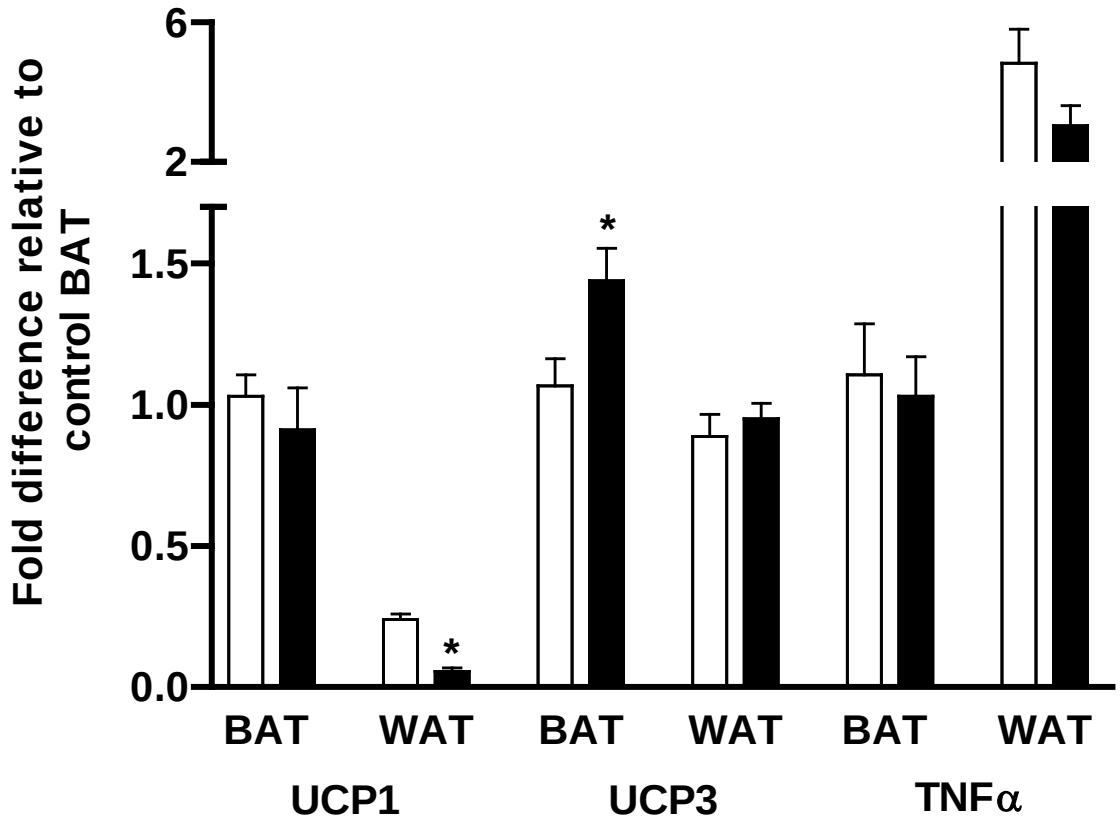

Fold difference relative to control BAT
6
2
1.5
1.0
0.5
0.0
*
*
BAT
WAT
BAT
WAT
BAT
WAT
UCP1
UCP3
TNFα

Fig. 3

Fig.1: a. Food intake (g chow/mouse/day) of control (open bar) and smoking (filled bar) Balb/C mice during experimental period. Mice were exposed to cigarette smoke or sham exposed from day 7 to 10. Results are expressed as mean ± S.E.M of 16 mice in each group.

* Significant difference between treatment groups ($P < 0.05$).

b. Body weight of control mice (open bar, n = 16) and smoking mice (filled bar, n = 16) during the experimental period. Mice were exposed to cigarette smoke or sham exposed from day 7 to 10. Results are expressed as mean ± S.E.M.

* Significant difference between treatment groups ($P < 0.05$).

Fig.2: NPY concentration of brain regions of control mice (open bar, n = 8) and smoking mice (filled bar, n = 7) after 4 days cigarette smoke exposure. Results are expressed as mean ± S.E.M.

Fig.3: UCP1, UCP3 and TNF α mRNA expression in BAT and WAT of control mice (open bar, n = 7) and smoking mice (filled bar, n = 8) after 4 days cigarette smoke exposure, standardized to 18s rRNA, expressed relative to a BAT sample from the control group (fold difference). Results are expressed as mean ± S.E.M.

* Significantly different from control group ($P < 0.05$).