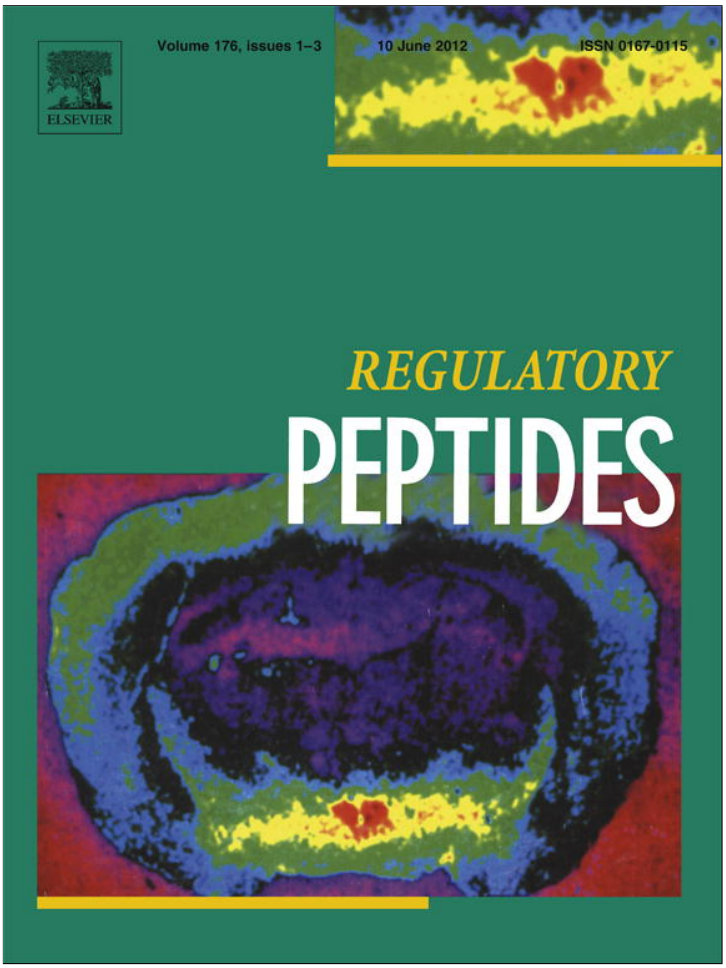

Regulatory Peptides 176 (2012) 22–27

Contents lists available at SciVerse ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep

Plasma neuropeptide Y levels relate cigarette smoking and smoking cessation to body weight regulation

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ARTICLE INFO

Article history:
Received 29 November 2011
Received in revised form 21 January 2012
Accepted 20 February 2012
Available online 28 February 2012

Keywords:
Neuropeptide Y
Leptin
Cigarette smoking
Smoking cessation
Body weight

ABSTRACT

Loss and disproportionate gain of body weight often seen respectively in smokers and quitters are believed to be due to disrupted energy homeostasis induced by nicotine, the major constituent of cigarette smoke. Energy homeostasis is suggested to be regulated by the coordinated actions of peripheral adipose tissue derived leptin and the brain hypothalamic orexigenic neuropeptide Y (NPY). While the studies probing the role of leptin and NPY in weight modulating effect of nicotine have so far been inconsistent and based largely on animal systems, there is a paucity of data involving human subjects. Here we measured the plasma levels of orexigenic neuropeptide Y (NPY) and leptin in 35 non-smokers and 31 cigarette smokers before and three months after smoking cessation. Compared to non-smokers, smokers were leaner and had reduced NPY and leptin levels. Smoking cessation resulted in a significant weight gain and increased waist circumference accompanied by increased leptin and NPY levels. NPY levels were significantly correlated with body weight ($r = 0.43$, $p < 0.05$), BMI ($r = 0.41$, $p < 0.05$), and waist circumference ($r = 0.37$, $p < 0.05$), while leptin correlated with BMI ($r = 0.42$, $p < 0.05$) and waist circumference ($r = 0.39$, $p < 0.05$). Association of leptin with smoking status, but not that of NPY, was lost after controlling for anthropometric parameters. Weight modulating effect of cigarette smoke may thus involve its direct action on NPY, independent of leptin. Altered leptin levels in smokers and quitters may merely reflect changes in body weight or precisely fat mass.

1. Introduction

Cigarette smoking is the leading preventable cause of death worldwide [1]. Despite increased public awareness of the detrimental effects of smoking, the number of active smokers remained significantly high throughout the world [2]. Smoking cessation programs aimed at helping people quit smoking have yielded mixed results owing to several negative attributes associated with smoking cessation. It is widely accepted that cigarette smoking lowers body weight and smoking cessation promotes weight gain, besides negatively affecting several psychological and physiological functions [3,4]. Such undesirable effects continue to deter smokers from quitting despite the benefit of smoking abstinence that overweighs smoking.

Optimum body weight is maintained by a balance between energy intake and expenditure, which is controlled by the coordinated actions of peripheral metabolic signals and several brain hypothalamic orexigenic and anorexigenic peptides [5,6]. Neuropeptide Y (NPY) is a 36-amino acid orexigenic neuropeptide with potent appetite stimulating properties [7,8]. NPY is synthesized by the neurons that extend from arcuate nucleus (ARC) into the paraventricular nucleus (PVN) in the hypothalamus and relays orexigenic signals [9,10]. It has been reported that central administration of NPY leads to obesity [11], while decreased hypothalamic NPY promotes diet-induced obesity [12].

Leptin, a critical peripheral anorexic hormone secreted by adipose tissue, governs food intake in the hypothalamus [13,14]. Leptin receptors are expressed in the hypothalamic regions of both humans and rodents [15,16]. Leptin crosses blood brain barrier via a saturable transport mechanism to interact with central pathways that regulate energy homeostasis [17,18]. Leptin has been reported to exert its anorectic effect by blocking AMP-activated protein kinase (AMPK) or activating acetyle-CoA corboxylase in the ARC and PVN regions of hypothalamus [19,20]. Chronic leptin administration reduces food intake and body weight and the reduction of central leptin signaling leads to hyperphagia and obesity [21,13]. However, chronic obesity

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0167-0115/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2012.02.005

often raises leptin levels corroborated with increased fat mass leading to reduced leptin sensitivity to their hypothalamic effects [22,23]. Leptin and NPY regulate energy homeostasis by feedback mechanism, where under positive energy balance, higher leptin levels inhibit hypothalamic NPY to suppress the appetite, while under negative energy balance lower leptin levels fail to exert inhibitory action on NPY thereby increasing the food intake [24–26].

Nicotine receptors are highly expressed in the hypothalamus and medulla [28]. Weight reducing effect of nicotine is principally achieved by suppressing the appetite and augmenting energy expenditure [28–31]. Mechanistically, nicotine and cigarette smoking have been shown to modulate body weight by interfering with NPY and leptin signaling [28,32,33]. However the studies have been inconsistent and are largely based on animal models. On the other hand, studies delineating the role of cigarette smoke on NPY in human subjects are very limited and beyond the context of body weight regulation. Thus, in this study we monitored the plasma NPY and leptin levels in relation to a change in smoking status with an aim to assess their roles in the body weight regulation in response to cigarette smoking and its cessation.

2. Materials and methods

2.1. Subjects

This Study was conducted in accordance with the guidelines set by the ethical committee, Research Center, College of Science, King Saud University. A total of 31 healthy Saudi chronic cigarette smokers who have been smoking for at least two years, and volunteered to quit smoking were recruited for the study and referred here as quitters. Non-smokers consisting of 35 healthy Saudi individuals were randomly selected from the RIYADH COHORT, a nationwide screening program for biomarkers, were recruited for the study. Informed consents were obtained from all the participating subjects. A structured questionnaire collecting the information on socio-demographic characteristics and present and past medical conditions was collected. Information on the smoking history comprising the duration of cigarette smoking, the number of cigarettes smoked per day and any discontinuity or a major change in the smoking behavior, were obtained. Smoking and non-smoking states of the subjects were confirmed by estimating the plasma cotinine levels. Fasting blood samples were collected from the control and smokers immediately before the cessation of smoking without a time gap of abstention and 3 months following the smoking cessation. Plasma was separated from the blood by Ficoll-Paque PLUS (GE health Care, Germany) gradient centrifugation. Plasma samples were stored at −80 °C until analyzed.

2.2. Anthropometric and biochemical measurements

Height and weight were recorded to the nearest 0.5 cm and 0.1 kg respectively. Waist and hip circumferences were measured using a standardized tape measure and was recorded to the nearest 0.1 cm. Body mass index (BMI) was calculated by the formula; weight (Kg) ÷ height (m^2). Plasma total-cholesterol and triglycerides were measured using standard enzymatic methods and a fully automated analyzer (Konelab instruments, Finland). HDL-cholesterol levels were determined by phosphotungstic acid/magnesium chloride precipitation (Kone instruments, Finland). LDL-cholesterol was calculated using Friedewald equation.

2.3. Leptin and NPY estimation

Plasma leptin levels were quantified using multiplex assay kits that utilize fluorescent microbead technology, allowing simultaneous quantification of several target proteins within a single plasma sample of 50–100 μL. These included pre-mixed and fully customized panels that utilize the Luminex® xMAP® Technology platform (Luminexcorp, TX, USA). Plasma neuropeptide Y levels were measured by ELISA following the manufacturer's instructions (Millipore, UK).

2.4. Statistical analysis

Data was represented by mean ± standard deviation. Skewed data was either log or square root transformed. One way Analysis (ANOVA) followed by Tukey's post hoc was used to test the differences among non smokers, smokers and ex-smokers. P value less than 0.05 was considered significant. Correlation analysis was carried out using Pearson correlation test. While comparing leptin and neuropeptide among smoker groups, Analysis of Co-variance (ANCOVA) was used to control for the possible confounders of BMI and Waist circumference. Adjustments for multiple comparisons were performed using the Bonferroni correction method.

3. Results

3.1. Anthropometric and biochemical parameters

Anthropometric and biochemical data are presented in Table 1. Smokers and non-smokers were all men and age matched. Smokers who volunteered to quit the smoking were referred as quitters in the present study. Compared at base line smokers were leaner than non-smokers (70.1 ± 12.3 vs. 77.9 ± 11.8 kg., p = 0.02). BMI (23.6 ± 4.5 vs. 27.8 ± 5.2 kg/m^2, p = 0.001), waist circumference (92.2 ± 18.0 vs.

Table 1
Anthropometric and biochemical parameters in non-smokers, smokers and quitters.

Parameters	Non-smokers (G1)	Smokers (G2)	Quitters (G3)	P-values	
				G1 vs. G2	G2vs. G3
N	35	31	31		
Age	48.1 ± 6.1	48.5 ± 9.3	48.9 ± 6.0		
BMI (kg/m^2)	27.8 ± 5.2	23.6 ± 4.5	26.3 ± 5.2	0.001	0.04
Weight (kg)	77.9 ± 11.8	70.1 ± 12.3	73.8 ± 11.1	0.02	0.04
WC (cm)	102.7 ± 17.3	92.2 ± 18.0*	98.3 ± 16.2	0.01	0.04
HC (cm)	103.6 ± 16.2	94.2 ± 12.0	98.6 ± 13.3	0.03	0.45
W/H ratio	1.1 ± 0.15	0.97 ± 0.10	1.0 ± 0.14	0.001	0.69
Total cholesterol (mmol/l)	5.2 ± 1.0	7.2 ± 1.9	6.7 ± 1.4	<0.001	0.33
HDL-cholesterol (mmol/l)	1.1 ± 0.31	0.80 ± 0.23	0.92 ± 0.29	0.001	0.04
LDL-cholesterol (mmol/l)	3.2 ± 1.0	4.3 ± 1.4	3.9 ± 1.2	0.006	0.37
Triglycerides (mmol/l)	1.4 ± 0.12	2.1 ± 0.16	1.9 ± 0.17	0.02	0.72

Data represented by Mean ± standard deviation, analysis of variance is done followed by Tukey's post-hoc test for comparison among groups. G1: non-smokers, G2; smokers, G3; quitters.

102.7 ± 17.3 cm, p = 0.01), hip circumference (94.2 ± 12.0 vs. 103.6 ± 16.2, p = 0.03) and waist–hip (W/H) ratio (0.97 ± 0.10 vs. 1.1 ± 0.15, p<0.001) were also significantly lower in smokers compared to non-smokers. Smokers had significantly increased total cholesterol (7.2 ± 1.9 vs. 5.2 ± 1.0 mmol/l, p<0.001), triglycerides (2.1 ± 0.16 vs. 1.4 ± 0.12 mmol/l, p<0.001) and LDL-cholesterol (4.3 ± 1.4 vs. 3.2 ± 1.0 mmol/l, p = 0.001) compared to non-smokers. In contrast HDL-cholesterol was significantly decreased in smokers than non-smokers (0.80 ± 0.23 vs. 1.1 ± 0.31 mmol/l, p<0.001). Smokers significantly gained body weight (73.8 ± 11.1 vs. 70.1 ± 12.3 kg, p = 0.04), BMI (26.3 ± 5.2 vs. 23.6 ± 4.5 kg/m^2, p = 0.034) and waist circumference (98.3 ± 16.2 vs. 92.2 ± 18.0 cm, p = 0.04) but not hip circumference after smoking abstinence for three months. HDL-cholesterol significantly increased (0.92 ± 0.29 vs. 0.80 ± 0.23 mmol/l, p = 0.04), while no change was found in total cholesterol, triglycerides and LDL-cholesterol following smoking cessation.

3.2. Altered leptin and NPY expression

To study the effect of smoking and smoking cessation on orexigenic brain hypothalamic neuropeptides and peripheral anorexic proteins, we measured the leptin and NPY levels in the studied subjects. Leptin and NPY levels are presented in Table 2. Compared to non-smokers, smokers were found to have significantly decreased leptin (22.4 ± 4.2 vs. 10.1 ± 2.3 ng/ml, p<0.001 respectively) and NPY (20.1 ± 2.2 vs. 17.2 ± 2.4 pg/ml, p<0.001) levels. Smokers who abstained from the smoking for three months exhibited remarkable changes in both leptin and NPY levels. Leptin levels significantly increased in quitters compared to smokers (19.2 ± 2.2 vs. 17.2 ± 2.4 ng/ml, p = 0.003 respectively). Likewise, NPY levels were increased in quitters as compared to smokers (16.3 ± 3.2 vs. 10.1 ± 2.3 pg/ml, p<0.001).

3.3. Leptin and NPY correlates with anthropometric parameters

In order to assess the correlations between leptin and NPY levels with anthropometric parameters including weight, BMI and waist circumference, we applied Pearson correlation analysis. Correlations of leptin and neuropeptide Y levels with anthropometric measures are provided in Table 3. NPY levels were significantly and positively correlated with body weight (r = 0.43, p<0.05), BMI (r = 0.41, p<0.05) and waist circumference (r = 0.37, p<0.05). Leptin levels on the other hand were significantly correlated with BMI (r = 0.42, p<0.05) and waist circumference (r = 0.39, p<0.05) and attained near significant correlation with body weight (r = 0.36, p = 0.05). However, none of the 3 anthropometric parameters retained the significance for correlations with leptin and NPY after Bonferroni correction at the new level of α set at 0.017 (0.05/3 = 0.0166).

We also evaluated the association of leptin and NPY with non-smoking and smoking states before and after smoking cessation after adjusting for weight and waist circumference to examine whether the changes in leptin and NPY levels reflect the effect of smoking or merely the resultant of weight gain. The data are presented in Table 4. Leptin levels were significantly low in smokers compared to non-smokers (p<0.001), and significantly high in quitters compared to smokers (P<0.001). However, the significance of differences in leptin levels between non-smokers and smokers was lost after adjusting for body weight (p = 0.07) and waist circumference (p = 0.06). Likewise, no significant difference was found between smokers and quitters after adjusting for body weight (p = 0.086) and waist circumference (0.081). On the other hand, NPY levels were significantly low in smokers compared to non-smokers (p<0.001) and significantly high in quitters compared to smokers (p<0.01) before controlling for weight and waist circumference. Importantly, NPY levels remained significantly different between non-smokers and smokers after adjusting for body weight (p<0.05) and waist circumference (p<0.05). Also differences in NPY levels between smokers and quitters sustained the significance after controlling for body weight (p<0.05) and waist circumference (p<0.05).

Table 2
Leptin and NPY levels in non-smokers, smokers and quitters.

	Non-smokers (G1) (N = 35)	Smokers (G2) (N = 31)	Quitters (G3) (N = 31)	G1 vs. G2	G2 vs. G3
NPY (pg/ml)	20.1 ± 2.2	17.2 ± 2.4	19.2 ± 2.2	<0.001	0.003
Leptin (ng/ml)	22.4 ± 4.2	10.1 ± 2.3	16.3 ± 3.2	<0.001	<0.001

Data represented by Mean ± standard deviation; analysis of variance is done followed by Tukey's post-hoc test for comparison among groups. G1: non-smokers, G2; smokers, G3; quitters.

Table 3
Pearson correlations between leptin, NPY and anthropometric parameters.

	Leptin (ng/ml)		NPY (pg/ml)	
	(r)	*P-value	(r)	*P-value
Weight (kg)	0.36	0.05	0.43	<0.05
BMI (kg/m^2)	0.42	<0.05	0.41	<0.05
WC (cm)	0.39	<0.05	0.37	<0.05

Pearson correlation coefficient is calculated. BMI: NPY: neuropeptide Y, Body mass index, WC: waist circumference. Level of significance is given at P ≤ 0.05. *Values rendered insignificant for correlations after Bonferroni correction with the new level of α set at 0.017 (0.05/3 = 0.0166).

4. Discussion

Disproportionate body weights in smokers and quitters are believed to be due to the ability of nicotine to destabilize energy homeostasis by influencing the expression of appetite regulating adipose tissue derived leptin and uncoupling proteins as well as several hypothalamic orexigenic and anorexigenic neuropeptides. There is a degree of disagreement in the studies dealing with the effect of nicotine on NPY and leptin expressions. Most of the studies were carried out in animal system and there is a dearth of studies focusing on human subjects and none dealing with the weight control. In this study we measured leptin and NPY levels in the plasma samples mainly to relate their expressions to body weight control in cigarette smokers before and after smoking cessation. We found significantly decreased body weight and lower levels of NPY and leptin in smokers compared to never smokers and significantly increased body weight and higher levels of leptin and NPY in quitters compared to smokers.NPY levels but not that of leptin remained significantly low in smokers and high in quitters even after controlling for body weight and waist circumference.

Consistent with a number of earlier reports [34–36], we found the smokers to have significantly lower body weights than non-smokers

Table 4
NPY and leptin levels before and after adjusting for body weight and waist circumference.

	Non-smokers (G1)	Smokers (G2)	Ex-smokers (G3)	G1 vs.G2 p-value	G2 vs.G3 p-value
N	35	31	31		
NPY (pg/ml)	20.1 ± 2.2	17.2 ± 2.4	19.2 ± 2.2	<0.001	<0.01
Model 1	20.6 ± 0.40	17.5 ± 0.53	19.0 ± 0.51	<0.05	<0.05
Model 2	20.3 ± 0.43	17.6 ± 0.56	18.9 ± 0.58	<0.05	<0.05
Leptin (ng/ml)	22.4 ± 4.2	10.1 ± 2.3	16.3 ± 3.2	<0.001	<0.001
Model 1	21.3 ± 0.88	12.0 ± 0.51	15.6 ± 0.60	0.077	0.086
Model 2	21.2 ± 0.76	12.6 ± 0.63	15.4 ± 0.62	0.06	0.081

Data represented by Mean ± standard deviation; analysis of variance is done followed by Tukey's post-hoc test for comparison among groups. Models 1 and 2 represented by Mean ± SE Model 1 controlled for body weight; Model 2 controlled for waist circumference. NPY: neuropeptide Y.

and to gain weight after quitting the smoking. Although in the present study we did not measure the changes in fat mass, the increased body weight after smoking cessation could possibly be due to increased body fat as waist circumference values of quitters are higher than those measured before giving up the smoking [37]. Numerous mechanistic studies have examined the role of NPY towards understanding the causal relationship between nicotine use and the changes in body weight [28,32,33,38,39]. In the present study, lower body weight accompanied with decreased NPY levels in smokers compared to non-smokers suggests that weight reducing property of cigarette smoke is mediated by its direct inhibitory action on orexigenic NPY, which may eventually results in appetite suppression and weight reduction. Substantiating this, we found the reversal of these effects after smoking cessation, where we observed increased body weight with a concomitant increase in NPY. Further, positive correlations between NPY and anthropometric parameters in smokers and quitters and retaining the significance of these correlations even after controlling for body weight and waist circumference underscore the role of this orexigenic peptide in weight modulation. To the best of our knowledge there are only two studies that have measured plasma NPY levels in human smokers and none in ex-smokers [40,41]. The decreased plasma NPY levels in smokers relative to non-smokers in our study are in contrast to these studies, where plasma NPY levels were found to be either elevated [40] or unchanged [41] in smokers. These discrepancies could have stemmed from the mode and the amount of cigarette smoke exposure. While we report the plasma NPY levels in chronic cigarette smokers, the previous studies have measured the NPY levels after allowing the subjects to inhale smoke from a single cigarette or two research grade cigarettes. Moreover, our study also differed with respect to ethnicity of the subjects involved and the methodology used to quantify plasma NPY content. Nevertheless, our data are in agreement with several animal studies, where cigarette smoke exposure or nicotine use has shown to downmodulate NPY and upon withdrawal to upregulate NPY levels. For example, acute (10–20 μg/rat) or chronic (4 mg/kg/day) nicotine administration through intracerebroventricular injection significantly decreased food intake and body weight in 1 h and 24 h respectively [42]. Nicotine at both situations significantly inhibited the NPY and these changes were antagonized by NPY receptor blocking, while the withdrawal of the receptor blockade led to increased NPY expression and weight gain indicating the involvement of NPY in regulating body weight. Likewise, nicotine intake at the concentration of 0.32 mg/mouse/day through drinking water for 16 weeks significantly decreased body weight and upon nicotine withdrawal led to a marked increase in body weight and NPY [43], whereas animals injected with nicotine (1 mg/kg body weight) twice per day for 2 days exhibited significant weight loss and decreased NPY levels [32]. Nicotine administration at 12 mg/kg/day concentration through osmotic mini pumps also significantly downmodulated the NPY levels in rats [38]. Similarly, paraventricular hypothalamic injection of NPY increased feeding and body weight and the effects were reversed by nicotine treatment elucidating a causal pathway linking nicotine, NPY and body weight [44]. Also, mice exposed to cigarette smoke (4 wk; 1 cigarette, 3×/d, 5 d/wk) showed marked decrease in body weight and NPY [28]. Contrasting with the above data, several studies have found unaltered or increased NPY levels after nicotine treatment or cigarette smoke exposure. Fourteen days of nicotine treatment at 6 mg/kg/day regimen significantly reduced food intake and body weight and increased the NPY mRNA and peptide levels [33]. Additionally, nicotine at 0.25, 1.5, and 3 mg/kg doses significantly decreased the body weight and upregulated the NPY mRNA levels in neonatal rat pups [39], whereas three cigarettes thrice daily for four days fail to induce any change in NPY levels [45]. It is apparent from above enumerated studies that they differ to a great extent on the duration of nicotine treatment or smoke exposure, amount of nicotine used or quantity of smoke exposed, and the differences in passive or active administration regimen. Therefore, possibility of any of these variants interfering with NPY expression cannot be ruled out [46]. Though in this study lower body weights in smokers paralleled reduced NPY levels, the possibility of other orexigenic or anorexigenic neuropeptides or the mechanisms beyond the purview of these regulatory peptides contributing these relationships cannot be ruled out. For example, nicotine at concentrations similar to those found in cigarette smoke is able to excite anorectic POMC neurons in the hypothalamus and reduce the body weight, linking this neuropeptide to weight loss associated with smoking [47]. Nicotine at varying doses, upregulated AGRP and POMC mRNAs and had negative effect on the body weight [39]. Further, nicotine withdrawal upregulated the AGRP along with the NPY and increased the body weight after smoking cessation [43]. Anorectic CART expression is augmented in nicotine treated mice, which is correlated with anorexia and weight loss [9,48,49]. Effect of nicotine or cigarette smoke on body weight may also be mediated by altered brown and white adipose tissue expression of uncoupling proteins (UCPs), which promote increased or decreased energy expenditure respectively after nicotine exposure or withdrawal [28,31,43,50].

Leptin is a major adipose tissue derived protein and its levels are directly proportional to adipose tissue mass [51]. Under positive energy balance leptin levels are suggested to be increased to inhibit the brain hypothalamic orexigenic neuropeptides to suppress the appetite, while in circumstances of negative energy balance its levels are believed to be decreased [13,14,18,25,26]. Accordingly, leptin levels are expected to be low in smokers and high in quitters. Several studies have measured the circulating leptin levels to assess its role in nicotine induced changes in body weight. However, the data are ambiguous with several studies reporting increased plasma leptin levels [39,52–57], while others have found decreased or unaltered levels [35,45,58–62]. In the present study we found reduced levels of leptin in smokers and increased levels in quitters, which may be in agreement respectively of decreased and increased body weights as the leptin levels were positively correlated with BMI and waist circumference in both smokers and quitters. Correlation of leptin with body weight also reached to a near significant value ($p = 0.05$) but fell short of significance. It is likely that muscle mass of subjects, which also contributes to body weight measurement along with the fat mass could have interfered with the correlation analysis resulting in the loss of significance between leptin and body weight. In this study, the significance of leptin association with the body weight in smokers and quitters was lost after adjusting for anthropometric parameters, indicating the unlikely participation of leptin in weight regulation in response to cigarette smoking and changes in leptin levels in smokers and quitters may merely coincide with changes in body weight or precisely change in the fat mass.

Given the established function of leptin, decreased leptin levels in smokers and increased levels in quitters found in the study were expected to promote the weight gain and to blunt the body weight increase respectively. This is in contrast to measured lower body weights of smokers and higher weights of quitters. Thus, it is likely that cigarette smoke may directly exert inhibitory action on NPY or other orexigenic peptides to suppress the appetite and promote weight loss independent of leptin. Accordingly, smoking cessation may trigger the weight gain as a result of withdrawal effect of nicotine on feeding peptides irrespective of leptin levels. Consistently, plasma leptin levels fell significantly only in lean nicotine-treated animals, whereas no change was observed in obese nicotine-treated animals. However, both lean and obese nicotine-treated animals had similar reductions in body weight indicating the leptin independent effects of nicotine in lowering the body weight [63]. Also, nicotine treatment reduced the body weight, mRNA levels of feeding peptides and increased the leptin levels in rat pups, whereas blocking the hypothalamic nicotine receptors blunted the nicotine induced changes but had no effect on leptin levels indicating the leptin independent effect of nicotine on body weight [39]. In line, nicotine has been shown to affect body weight independent of leptin [50,61]. In contrast

several studies have indicated the independent effect of nicotine on leptin expression [33,52–56].

5. Conclusions

The findings in the present study of lower body weight, with an accompanying decrease in NPY in smokers and increased body weight with an accompanying increase in NPY in quitters imply that weight modulating effect of cigarette smoke is likely mediated by its direct action on NPY independent of leptin. Changes in leptin levels in smokers and quitters may simply correspond to the change in the body weight. Our data also provide an insight in to the ethnicity based variations in the levels of NPY and leptin in response to cigarette smoke exposure and its withdrawal. The data thus suggest that ethnicity might partly contribute to variations in the relationship between NPY and leptin and the body weight modulation.

Author contribution

TH and NMA; designed and conceived the study, participated in preparing the draft. OSA; helped design and conceive the study, analyzed and interpreted the data and critically evaluated the draft. HMD and SMY; carried out the biochemical measurements and obtained the data. SHA; participated in statistical analysis, drafted the manuscript and helped analyze and interpreted the data. All authors declare the submission and approval of the manuscript.

6. Conflict of interest

All authors disclose non-existence of potential conflict of interest including any financial, personal or other relationships with other people or organizations.

Acknowledgements

This work was funded by the King Abdulaziz City for Science and Technology (KACST) Research Grant, LGP-14-22. Authors thank Abdul Khader, Usamah Kazmi, Mohammed Ghouse Ansari for technical help and Benjamin Vinodson for statistical analysis.

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