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No Change in Serum Incretins Levels but Rise of Leptin Levels After Smoking Cessation: a Pilot Study

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Summary

The mechanisms behind the changes of body weight after smoking cessation are only partially understood. To this end, we explored the possible effects of smoking cessation on incretin hormones, leptin and selected anthropometric, biochemical and other hormonal parameters. Twenty-two non-obese male adult smokers attending an ambulatory smoking cessation program in Prague, Czech Republic, were examined at the baseline. Thirteen patients (mean age 37.92±2.66 years, mean body mass index 25.56±0.69 kg/m^2) successfully quit smoking and were examined three months after smoking cessation; relapsed smokers were not followed up. The patients underwent 2-h liquid meal test with Fresubin and repeated blood sampling for measurements of blood glucose, gastric inhibitory polypeptide (GIP), glucagon-like peptide 1 (GLP-1), amylin, insulin, leptin, peptide-YY (PYY) and pancreatic polypeptide (PP). Three months after smoking cessation, body weight increased (4.35±3.32 kg, $p<0.001$). Leptin levels increased significantly in all repeated samples, while levels of GIP, GLP-1, amylin, insulin, PYY and PP remained unchanged. In conclusions, smoking cessation increased leptin levels probably owing to weight gain while it did not influence incretin levels.

Key words

Smoking cessation • Body weight • Leptin • Glucagon-like peptide 1 • Glucose-dependent insulinotropic peptide

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Introduction

Incretins are hormones secreted in the gut in response to food ingestion (Nauck 2011) as well as in the hindbrain (Egecioglu *et al.* 2013) and help to manage glucose control by regulating insulin and glucagon release (Seino *et al.* 2010, Madsbad 2014), gastric emptying (Edholm *et al.* 2010, Deane *et al.* 2010, Meier *et al.* 2006, Schirra *et al.* 2006) and calorie intake (Williams *et al.* 2009, Peters 2010, Woods 2005). Two incretin hormones – glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (Baggio and Drucker 2007) regulate islet hormone secretion, glucose concentrations (Campbell 2011) as well as lipid metabolism (Meier *et al.* 2006, Phillips and Prins 2011, Zinman *et al.* 2009), gut motility (Campbell and Drucker 2013), appetite and body weight (Flint *et al.* 1998, Flint *et al.* 2000, Dirsen *et al.* 2012, Verdich *et al.* 2001), and immune function (Campbell and Drucker 2013). GLP-1 modulates food intake and body weight *via*

GLP-1 receptors (GLP-1R) expressed in the hypothalamus (McMahon and Wellman 1998) and in the nucleus tractus solitarius that project throughout the brain to areas such as the hypothalamus and mesolimbic areas (Alvarez *et al.* 1996, Mechenthaler *et al.* 1999, Holst 2007, Baggio and Drucker 2007, Hayes *et al.* 2009). Additionally, GLP-1R are expressed in several brain areas such as the reward nodes ventral tegmental area and nucleus accumbens (Alvarez *et al.* 1996, Mechenthaler *et al.* 1999), implicating that GLP-1 may have a role in reward regulation. The findings showing that activation of GLP-1R in these areas reduces the intake of highly-palatable foods in rodents (Alhadeff *et al.* 2012), suggest that these receptors may be involved in stimulation of the mesolimbic dopamine system. Novel evidence shows that GLP-1R regulates nicotine-induced activation of the mesolimbic dopamine system in mice (Engel and Jerlhag 2014). It has been also confirmed that GLP-1 controls reward induced by alcohol, amphetamine and cocaine (Engel and Jerlhag 2014) and that GLP-1 could serve as a potential novel treatment target for several drug addictions. GIP and GLP-1 are secreted from the L-cells of the lower gut and K-cells of the intestines (Kreymann *et al.* 1987, Holst 2007, Mortensen *et al.* 2003), respectively, and their production and action are reduced in patients with type 2 diabetes mellitus (Azimova *et al.* 2014, Sala *et al.* 2014). In majority of patients with type 2 diabetes mellitus, the incretin system dysfunction is manifested by a lack of rise of GLP-1 after food ingestion (Meier and Nauck 2008, Vollmer *et al.* 2008) and reduced sensitivity of pancreatic β-cells to the effects of GIP and partly GLP-1 (Nauck *et al.* 1993, Jones *et al.* 1989).

Incretin-based therapies are nowadays widely used in the treatment of type 2 diabetes mellitus. The goal of these therapies is to increase GLP-1 effects and there are two groups of these ones: glucagon-like peptide-1 receptor (GLP-1R) agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors. The use of GLP-1R agonists leads to a significant reduction in blood glucose and glycated hemoglobin due to increased stimulation of insulin secretion and decrease of glucagon levels (Bose *et al.* 2009, Haluzík *et al.* 2014*)*. Inhibition of dipeptidyl peptidase-4 (DPP-4), the enzyme responsible for N-terminal cleavage and inactivation of GIP and GLP-1 (Drucker 2003, Deacon 2004), leads to increased insulin secretion, reduced glucagon secretion, and lower glucose concentrations (Drucker 2011).

As described above, incretin hormones are important regulators of glucose metabolism and energy balance. For example: increased GLP-1 concentrations have been found after certain types of bariatric surgery and this increase has been implicated in the body weight independent metabolic effects of bariatric surgery (Bose *et al.* 2009, le Roux *et al.* 2007, Vetter *et al.* 2009, Falken *et al.* 2011). GLP-1R agonists consistently decrease body weight not only in patients with diabetes but also in obese non-diabetics (Iepsen *et al.* 2014). In fact, one of the GLP-1R agonists' liraglutide has been recently approved for the treatment of obesity. Smoking has been associated with lower body weight (Albanes *et al.* 1987, Molarius *et al.* 1997) while smoking cessation frequently causes body weight gain (Klesges *et al.* 1989, 1997, Aubin *et al.* 2012, Kmetova *et al.* 2014).

Leptin is a cytokine released from the adipose tissue and is known to suppress food intake by decrease of appetite after eating and increase the metabolic rate (Perkins and Fonte 2002). Leptin rises with food ingestion and modifies the balance between appetite stimulation and inhibition in the hypothalamus, through an action involving neuropeptide Y, leading to a consecutive decrease in food intake (Baskin *et al.* 2001, Schwartz *et al.* 1998, Mizuno *et al.* 1998). Several studies reported that leptin may be a potential mediator of weight gain following smoking cessation (Hodge *et al.* 1997, Chen *et al.* 2006, Koopmann *et al.* 2011). On the other hand, these differences between smokers and non-smokers disappear after adjusting for body mass index (Nicklas *et al.* 1999).

We hypothesized that smoking may increase serum incretin levels (in particular GLP-1) and serum leptin levels which may contribute to reduced food intake in smokers and conversely to weight gain after smoking cessation. To test this hypothesis, we measured serum incretin and leptin levels, selected gastrointestinal hormones and other relevant metabolic parameters in smokers before and after smoking cessation.

Methods

Study subjects

22 non-obese male adult smokers planning smoking cessation in the Centre for Tobacco-Dependent in Prague, Czech Republic, between 2013 and 2014 were enrolled in this study. Out of these, 13 successfully quit smoking (mean age 37.92 years, SD 9.99, range 27-59 years) and only these patients were used for further analyses. Except of patients‘ statement to be smoke-free,

smoking status was biochemically verified by carbon monoxide (CO) measurement in expired air, cut-off <6 ppm, Smokerlyzer Micro+ device (Bedfont, Kent, UK), both performed at each visit.

None of the subjects suffered from diabetes (defined as fasting plasma glucose >7.0 mmol/l) or impaired fasting glucose (between 5.6 and 6.9 mmol/l). Also, none of the patients used any form of nicotine during the three-month period after smoking cessation.

Written informed consent was signed by all participants before being enrolled in the study.

The study was approved by the Human Ethical Review Committee (FWA 00003027), First Faculty of Medicine, and the General University Hospital, Prague, Czech Republic, (IORG 0002175), registered under No. IRB 00002705 and was performed in accordance with the guidelines proposed in the Declaration of Helsinki.

Anthropometric examination and blood sampling

Successful quitters were examined at baseline and after three months of continuous abstinence. All subjects were measured and weighed, and their body mass index (BMI) was calculated. Casual blood pressure (BP) values were obtained in the sitting position using a digital sphygmomanometer (M10-IT, Omron Healthcare Co., Kyoto, Japan). Blood samples for biochemical and hormonal measurements were withdrawn by repeated sampling at the baseline, and after 5, 15, 30, 60, 90 and 120 min, after ingestion of 200 ml Fresubin (a vanilla flavored liquid consisting of protein (milk), vegetable oils (rapeseed oil, sunflower oil), carbohydrates, vitamins, minerals and trace elements, gluten and lactose free, producer Fresenius Kabi, Bad Homburg, Germany) between 07:00 h and 10:00 h after an overnight fasting both at the baseline (last cigarette approximately 1 h before sampling) and after three months of continuous abstinence from smoking. Serum was obtained by centrifugation and the samples were subsequently stored in aliquots at −70 °C until further analysis.

Hormonal and biochemical assays

Serum amylin, leptin, peptide YY (PYY), pancreatic polypeptide (PP), insulin, GLP-1 and GIP were measured by commercial Luminex xMAP kit (Millipore, Merck Co., Darmstadt, Germany). Sensitivity was 11.0 ng/ml for amylin, 184.0 ng/ml for leptin, 26.0 ng/ml for PYY, 5.0 ng/ml for PP, 48.0 ng/ml for insulin, 20.0 ng/ml for GLP-1 and 0.7 ng/ml for GIP. Intra- and interassay variabilities for the kits were <10 % and 15 %, respectively.

HbA1c (glycated hemoglobin) was measured in the Department of Biochemistry of the General University Hospital by high performance liquid chromatography (analyzer Variant, Bio-Rad Co., Hercules, USA).

Blood glucose was evaluated by blood glucose test meter Glucocard X-meter and test stripes Glucocard X-sensor (both Arkray Co., Kyoto, Japan), and based on quantitative measurement by electrochemical method.

Tobacco dependence treatment program

All patients were treated by combination of psychobehavioral intervention and pharmacotherapy (varenicline, and/or bupropion) (Fiore *et al.* 2008, Kralikova *et al.* 2015). Pharmacotherapy choice was based on the therapist's recommendation (after a thorough assessment of the patient's history) and patient's choice. Due to possible influence of incretin metabolism, nicotine use was not allowed throughout the study and this was confirmed verbally during the interview at each visit.

At the baseline visit, level of tobacco dependence was assessed according to the Fagerström Test of Cigarette Dependence (FTCD) (Heatherton *et al.* 1991, Fagerström 2012), medical history was collected, and a basic physical exam was performed. At the second visit, physical dependence and psycho-social tobacco dependence were discussed during the 2-h intervention (structure described at http://www.slzt.cz/intervention-structure). Follow-up visits lasted 30 min on average. The first of these was planned within one or two weeks after the target quit day, then about once a month up to the third month of treatment. The last visit was performed 3 months post-quit date.

Statistical analyses

Statistical analysis was performed using SigmaStat software (SPSS Inc., Chicago, IL, USA). Results were expressed as means ± SEM. Differences in anthropometric, biochemical and hormonal parameters before and three months after smoking cessation were evaluated using paired t-test or Wilcoxon Signed Rank Test as appropriate. Statistical significance was assigned to $p<0.05$.

Table 1. Anthropometric and biochemical characteristics at the baseline and three months after smoking cessation (successful male quitters only, N=13).

Characteristic	Baseline visit	Visit 3 months post-quit	Significance (*)
Age (years)		37.92, SD 9.99	–
Weight (kg)	81.66 ± 3.22	85.97 ± 3.25	**p < 0.001***
Waist circumference (cm)	90.54 ± 2.35	94.31 ± 2.78	**p = 0.005***
Hips circumference (cm)	102.77 ± 1.76	106.23 ± 1.70	**p = 0.009***
WHR	0.88 ± 0.02	0.89 ± 0.02	p = 0.641
BMI (kg/m^2)	25.56 ± 0.69	26.67 ± 0.71	**p = 0.003***
Glycaemia (mmol/l)	4.90 ± 0.13	5.04 ± 0.09	p = 0.267
Glycated hemoglobin (mmol/mol)	34.23 ± 0.75	32.46 ± 0.70	**p = 0.015***
CO (ppm)	19.08 ± 2.90	1.54 ±0.39	**p < 0.001***
COHb (%)	3.04 ± 0.47	0.20 ± 0.06	**p < 0.001***

* p<0.05

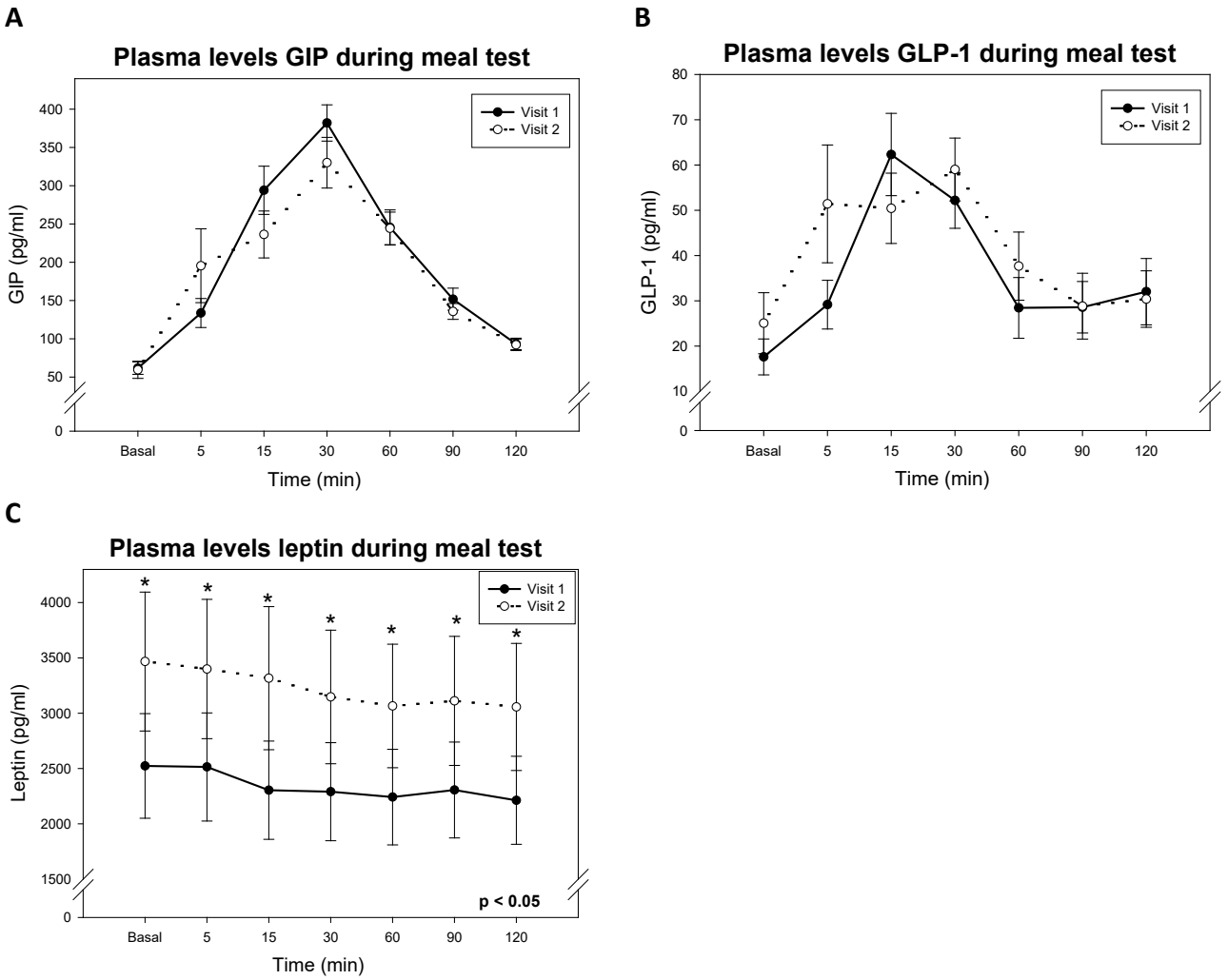

Fig. 1. Plasma levels of GIP (**A**), GLP-1 (**B**) and leptin (**C**) during 2-h liquid meal test with repeated sampling (baseline, 5th, 15th, 30th, 60th, 90th and 120th min) in the participants at the baseline (black, Visit 1, N=13) and after three months of smoking abstinence (white, Visit 2, N=13). Data are presented as means ± SEM. Differences between Visit 1 and Visit 2 were evaluated using paired t-test. * p<0.05

Results

Anthropometric and biochemical characteristics at the baseline and three months after smoking cessation are summarized in Table 1.

Three months post-quit, the body weight increased significantly, with the mean weight gain 4.35±3.32 kg (range 0.7-12.0 kg), $p<0.001$. Simultaneously, significant increase in BMI ($p=0.003$), both waist ($p=0.005$) and hips ($p=0.009$) circumference was recorded. Although there was no significant difference in repeated samples of blood glucose at the baseline and three months post-quit, small but significant decrease in HbA1c levels was noticed ($p=0.015$).

Serum incretin levels (e.g. GLP-1 and GIP) in all repeated samples were not affected by smoking cessation (see Fig. 1), as well as levels of amylin, PP and PYY. Increased leptin levels in all repeated samples after three-month smoking abstinence were found (the mean baseline leptin level was 2523.33±472.83 pg/ml at the first visit and 3466.84±627.82 pg/ml after three-month smoking abstinence), see Figure 1.

Discussion

The most important finding of our study is that serum incretin levels measured after meal challenge were not affected by smoking cessation. This finding argues against our hypothesis that smoking may increase serum incretin levels which would in turn contribute to lower body weight in smokers. Serum incretin levels in smokers have not been thoroughly studied previously. In the only published paper on this topic to date, only fasting serum GLP-1 concentrations were measured with no significant difference in its concentrations before and after smoking cessation (Stadler *et al.* 2014). GIP or other relevant gastrointestinal hormones have mostly also not been systematically measured in connection with smoking cessation.

Similarly to incretin hormones, smoking cessation did not influence levels of amylin, PP and PYY. No data about amylin or PP levels after smoking cessation have been available until our study. Similarly to our data, one study have previously found no change in fasting PYY levels after more than three months smoking abstinence (Stadler *et al.* 2014), while another work focused on PYY levels 24-48 h since the quit attempt and their association with craving and smoking relapse (al'Absi *et al.* 2014). The fact that we did not see significant changes in incretin or other gastrointestinal hormones levels three months after smoking cessation does not completely rule out their possible role in the regulation of energy homeostasis under these conditions. It is still possible that, similarly to PYY in the above described al'Absi´s paper, changes of incretin levels earlier after smoking cessation may have occurred.

Another interesting finding of our study is a significant increase in leptin levels after smoking cessation. Already published data about changes in leptin levels with regards to smoking cessation are rather inconsistent. Some papers described increased leptin levels after smoking cessation (Perkins and Fonte 2002, Lee *et al.* 2006, Hussain *et al.* 2012) with simultaneous report of post-cessation weight gain (Wing *et al.* 1996). Possible explanation of leptin levels increase accompanied by post-cessation weight gain is that smoking itself, *via* nicotinic mechanisms, may modify the sensitivity of hypothalamic leptin receptors and consequently modulate leptin synthesis, thus leading to body weight reduction (Hodge *et al.* 1997).

On the other hand, another publication did not find an increase in leptin levels following smoking cessation despite the weight gain (Stadler *et al.* 2014).

In our study, all of the successful quitters (N=13) gained weight and thus increased their BMI, waist- and hips circumference which is in agreement with previously published data (Aubin *et al.* 2012, Kmetova *et al.* 2014). Although no significant effect of smoking cessation on fasting or postprandial blood glucose levels was observed in our study, we have found the significant decrease in glycated hemoglobin levels. These findings are in line with some of previous studies (Cohen and Bellucci 2010, Clair *et al.* 2011, Soulimane *et al.* 2014) while other paper found no change in HbA1c (Kato *et al.* 2014). The reason for a slight improvement of HbA1c in our study is unclear, there is a possibility that the difference was found by chance.

There are several limitations of this work. As this is the pilot research, no power analysis was performed, as well as randomization. Due to possible interference of estrogens, female participants were excluded from this study and results thus cannot be generalized. In addition, it is important to point out that as this pilot study was performed with 13 patients only, results can be affected by an error of a small sample size.

In conclusion, our pilot study has demonstrated that three months of smoking abstinence increase body weight and serum leptin levels, decrease HbA1c and does

not significantly influence fasting or postprandial concentrations of GLP-1, GIP, amylin, PP and PYY. These findings suggest that incretin hormones do not appear to be involved in smoking-related changes in food intake and energy metabolism.

Conflict of Interest

There is no conflict of interest.

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